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ACTA OTO LARYNGOLOGICA
Vol 83 No 1 2

COMPTE RENDU
DE LA REUNION SCIENTIFIQUE DU

COLLEGIUM OTO-RHINO-LARYNGOLOGICUM

AMICITIAE SACRUM

STOCKHOLM LE 15 18 AUGUST 1976

GREV MAGNIGATAN 18 114 55 STOCKHOLM

50 YEARS OF THE COLLEGIUM OTO-RHINO-LARYNGOLOGICUM AMICITIAE SACRUM

Dear Members of the Collegium
Dear Friends

Fifty years, five decades, half a century, a fraction of a second of eternity or just two generations ago the Collegium Oto-Rhino-Laryngologicum was founded in Groningen in The Netherlands

It was a memorable period, only eight years after the end of the First World War, but rather like the present time, an era of instability The horrors of the war were not forgotten, hate persisted but the first beginnings of a better understanding between the warring nations had already begun Briand and Stresemann had met in Locarno and a political *rapprochement* had started

The seeds of peace sown in Locarno and the hope of better understanding between nations had its best chance of growth in the soil and limate of a neutral country like The Netherlands The Dutch scientists had the great advantage of freedom of travel between unfriendly nations They understood and spoke the necessary languages and acted as messengers of goodwill In addition they possessed a particular advantage in the field of otorhinolaryngology for in the Low Countries, such men as Zwaardemaker, Quix, Benjamins, de Kleijn, Versteegh, to name just a few, had connections in Austria, Belgium, Britain, France, Germany and Scandinavia Not only were they respected for their professional reputation but they had many good friends in these countries However, they felt unhappy, for they were aware that good scientific work can only prosper where there is possibility of a full exchange of ideas both nationally and internationally At this time unfortunately, international contacts were primarily within the two previously belligerent fractions and meetings were almost invariably restricted to clinical and surgical discussions Scientific subjects were sorely neglected but fortunately this prompted Benjamins and de Kleijn to write to a group of personal friends in 14 countries in Western Europe It was the moment of conception with a mercifully short period of gestation, only 3–4 months! The Collegium was born on the 8th October, 1926 (Fig 1) and immediately baptised Collegium ORL Amicitiae Sacrum Its Godfather was Burger, who stated quite clearly—and this must never be forgotten —"that one of the most important virtues of the Collegium is that its scientific meetings should be well organised, that good papers and discussion are of paramount importance but, most of all, that members should have their feet together under the table after the meetings" Perhaps this was the most important part of Collegium—friendship—for although it wasn't always the same table, bus or boat, the cordial atmosphere both before, during and after the scientific meeting has made the Collegium a centre of international harmonization, co-operation and certainly friendship How grateful we are for the wisdom of our founders in creating the Collegium under the flag of friendship

However, we must not underestimate the difficulties experienced by these wise men, many of their colleagues were opposed to any form of fraternization and hate and antagonism persisted in most countries Still, nothing could stop the development of the Collegium Amicitiae Sacrum It's true of course that not every country joined immediately, 34 founder-members were present (Fig 2, Table I) at the [illegible] sion and they came from nine [illegible]

Dear Colleague,

At the meetings of the oto-rhino-laryngological societies and at the international congresses the subjects dealt with are of widely divergent character The purely scientific subjects often do not receive sufficient attention chiefly on account of the following reasons

1 by the great number of papers read one's attention is distracted and too short a time is allowed for each speaker
2 of a number of members the interest in purely scientific subjects is only slight

We have therefore decided to invite a number of colleagues whom we know to take an interest in scientific problems in order to start an international Collegium Oto Rhino-Laryngologicum in which purely scientific problems will be discussed These may be of a theoretical, experimental, or clinical character, provided that the purely scientific aspect of the problem is predominant The membership of such a society must necessarily be limited, the number of papers read must also be limited in order to allow at least half an hour for each paper In some instance as much as an hour might be advisable in which case consent from the board would have to be obtained

In this way it will be possible to pay due attention to each problem and the presence of a number of people working in the *same direction should promote a very fertile discussion*

The language used would be English, French or German so that the majority of those present would be able to understand each other, thus we think that it would be possible to invite representatives from the following countries Austria, Belgium, Czecho-Slovakia, Denmark, Finland, France, Germany, Great Britain, Holland, Hungary, Norway, Spain, Sweden and Switzerland

We should be greatly obliged if you would send us the names of others in your country who are, in your opinion, suitable to receive an invitation We should like to remind you here that limitation in the number of members is advisable If we find sufficient response among our colleagues, we intend to convoke meeting in Groningen in October or November of this year, at ich a scheme worked out by us on the above lines and previous circulated will be discussed, the Society properly constitut-d, and a number of scientific lectures delivered

If the project proves successful, meetings might be held yearly, each time in one of the participating countries

Sincerely yours,

C E Benjamins
A de Kleijn

Fig 1 Letter written in July 1926 by Benjamins and de Kleijn to a group of leading oto-rhino laryngologists in Europe and the United States This letter led to the foundation of the Collegium English translation from German

this baby grew up quickly and on the 1st August, 1927, there were 81 members from 15 countries, including the United States of America Only one of these founder members is still alive and active Georges Portmann Active not only as a Collegium member but in the field of political harmonization he exemplifies the spirit of 50 years of the Collegium and how happy we all are that we can still listen to his stories of the early days of the Collegium It is also of enormous importance that my predecessor as General Secretary has written a history of the first 40 years of the Collegium ORLAS Though he passed away before this exceptional meeting, his history remains a memorial to both the man and the Collegium he loved

You have asked me to speak about the past 50 years of the Collegium and my task is made easy by the writings of Eelco Huizinga When World War II started, the Collegium was firmly established and thus survived this holocaust helped undoubtedly by the deep friendship between its members thus proving that Amicitia Sacrum was not just a pair of words The meeting of 1939 planned for Brussels was cancelled and it took 8 years before it was eventually held Many members had died One of these was Ledoux who should have been the Pre



Fig 2 The founding members of the Collegium ORLAS at the meeting in Groningen

dent in 1939 In 1947 a small group of members met informally but it took three more such informal meetings before the first post-war ordinary session of the entire Collegium was held in Zurich in 1952 under the Presidency of Ruedi This was the rebirth of the Collegium proper

If one is to describe the history of a Society he must not confine himself entirely with the details of conception, birth and rebirth without considering some facts

Up until the time of the Second World War the yearly fee was 10 shillings After the war it changed from Sterling to Dollar to French franc Naturally it increased—to 50 French francs, and one day it will probably increase again[11]

The number of papers at the various meetings has remained remarkably constant, somewhere between 30 and 35 Eelco Huizinga tells us that before the war the percentage of papers on the ear, its infections and diseases, was 67 This remained the same during the 10 post-war meetings but during the last 8 meetings has risen to 71 % for the ear This has been due to the great increase in interest in the field of hearing, equilibrium, anatomy and electron microscopy together with the considerable contribution of laboratory researchers in these subjects Once again we see that where the scientists lead, the clinicians follow and where the clinicians discover problems, the scientists enquire

Symposia have been held in Rome (on Standardization of Vestibular tests, 1949), Padua (the Physiological Mechanisms of the Vestibular Receptor, 1960), Lyon (the Sensorial Cell of Corti's Organ) and now in Stockholm (1976) we shall have two Symposia, one on Physiology and Pathophysiology of the Extrathoracic Airways Including the Middle Ear and one on The Normal and Pathological Structure and Function of the Inner Ear

Some things are part of the very fibre of the Collegium, others have become part of it dur-

Table II *The Shambaugh prize for otology*

1949 G von Békesy	1963 A C Hilding
1951 R Caussé	1965 G Dohlman
1953 H Davis	1967 H Engstrom
1955 C S Hallpike	1969 C Fernandez
1957 G Wever	1971 L B W Jongkees
1959 J R Lindsay	1973 G E Shambaugh, Jr
1961 L. Ruedi	1975 H Spoendlin

the cities where we meet or who live in countries where permission is refused to travel to the meetings are in no real danger of expulsion

I know of some very pleasant local meetings of members in both North and South America These help to foster the spirit of the Collegium and I have been fortunate enough to attend some of these

Financial problems do not always allow members to travel long distances or cross oceans to attend each and every meeting Others have University or other commitments which prevent attendance Most excuse themselves in personal letters to the President or General Secretary, for which we are grateful

There is however, one matter on which I can- remain silent, for it may threaten the very sis on which the Collegium has been built Even in 1926 the number of ENT meetings was large, now in 1976 it is considerably greater and the large number of papers read prevents any real in depth consideration or adequate time for discussion Many of the participants are not especially interested in scientific subjects—that is why the Collegium was founded Today we have another difficulty In 1926 most of the anatomical, physiological and histological studies relating to ENT were performed by clinicians The increasing sophistication of research techniques has led to this research being carried out now in specialized laboratories and the clinician finds himself with neither the time nor the expertise to compete in these fields of endeavour It is therefore even more important that there is good communication between scientists active in the spheres of fundamental research and those clinicians interested in these problems The clinician may have difficulty in understanding the somewhat complex and m tic language of the research worker and isolat from clinical problems will be to the disadv tage of the laboratory worker Both can le from each other and from the papers presen at the Collegium meetings, for this is one the few forums where both groups can m and talk freely Without such rapport the C legium cannot prosper I wish us all good lu

In the past, all members and senior memb were free to submit papers as long as the stracts were received by a fixed date All had right to have their contributions considered publication Despite the fact that rejection papers judged to be of insufficient scientific i portance might occur, the number of pap presented left little time for discussion

It might be a worthwhile task for my succes to consider means of solving this problem P haps more papers by invitation or round ta discussions, a fixed topic for the meeting or e a limit on the number of accepted papers T members will have to decide one day for the selves Despite all this I must state quite ca gorically that the Collegium has been for me best thing in my professional life as a clini ENT Surgeon and Research Worker Let never forget that most of the best things in c field of work have been conceived in the labo tory and in some instances, this laboratory w nothing more nor less than an easy chair, pea and a clear head that had learnt how to thi critically And let us also remember that problems that led to the research work oft came initially from the clinician Clinical wo is clinical and research work is research and ways the twain shall meet The ideal meeti point for ENT is within the Collegium duri after and also between meetings We have go

Table III *Permanent members of the committe*

1960	Leonard Jongkees	General Secretary
1963	Carl Axel Hamberger	Second Secretary
1966	Michel Portmann	Treasurer
	Michele Arslan	Councillor
1974	Don Harrison	Councillor
	Carl Rudolf Pfaltz	Deputy Secretary

able friends all over the world and I am sure that many of these friendships were made at Collegium meetings Together we are preparing the ground for the next generation We have a responsibility to them to preserve and strengthen the unity and harmony that exists within the Collegium This we must preserve, for it is precious in this time of disharmony and without it the spirit of the Collegium might die Thank yo[illegible] rusted your General Secreta[illegible] years [see Table III] Give your tru[illegible] mpathy to my successor and join me [illegible] ing words

Long Live the Collegium Oto-Rhino-Laryngologicum Amicitiae Sacrum

L B W Jongkees

Stockholm August 16 1976

SYMPOSIUM

Physiology and Pathophysiology of Extrathoracical Airways Including Middle Ear

Moderator S Ingelstedt

Mr President,
Members of the Collegium,
Ladies and Gentlemen,

It is a great honour and pleasure for us to present to this distinguished assembly some of the research work going on in Sweden today The subject is the Physiology and Pathophysiology of the Extrathoracic Airways [1] For many reasons I think it is warranted to include the middle ear in the respiratory tract, especia since established techniques for studying r spiratory physiology can be applied to investi tions on the function of the middle ear, as y will hear

[1] Pages 11–39

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EFFECTS OF MIDDLE EAR PRESSURE ON THE INNER EAR

Ö Tjernström

From the Department of Otolaryngology, University of Lund Malmö General Hospital, Malmö, Sweden

stract It was demonstrated in experiments on normal jects that moderate ambient pressure changes, creat ; overpressure in the middle ear, may induce a vestibu reaction In other experiments on subjects suffering m acute attacks of Meniere's disease, relief of symp- ns was achieved by means of ambient pressure changes the same magnitude

hen a subject is exposed to reduction of the nbient pressure and told not to equilibrate the iddle ears actively, the reduction will induce a lative overpressure in the middle ear When is relative overpressure has reached a certain vel, varying from one individual to another, e Eustachian tube will be forced open

When conducting such experiments, we found at some subjects reported vertigo and ENG vealed a simultaneous nystagmus (Ingelstedt t al, 1974) These subjects were found to have high forcing pressure, i e the Eustachian tube id not open passively until the relative over ressure in the middle ear was 60 cm H_2O or nore Fig 1 (above) shows a middle ear model vith a relative overpressure owing to reduction of the ambient pressure When this relative verpressure reached about 60 cm H_2O, a ves ibular reaction was elicited (Fig 2, above) Repeated examinations at intervals of years al ways gave the same results—vertigo and nystag- mus It should also be mentioned that the sub- jects with a high forcing pressure had a quite normal capacity for active middle ear pressure equilibration About 7% of normal subjects were found to have such high forcing pressure levels

This paper was prepared together with Professor Sven Ingelstedt and Engineer Alf Ivarsson

In other experiments subjects with a high forcing pressure were exposed to overpressure applied to the middle ear direct via an eardrum perforation (cf Fig 1, below) Fig 2 (below) demonstrates that even in these experiments nystagmus started when the pressure level reached about 60 cm H_2O (Tjernström, 1974) Underpressure applied to the middle ear did not induce any vestibular reactions Thus, ear- lier proposed mechanisms, such as fistulas to the inner ear (Barany, 1908), thin bony walls between the middle and the inner ear (Melvill Jones, 1957, Benson, 1965) might be excluded in these cases The experiments also exclude sudden stapes movements (Melvill Jones, 1957, Fields, 1958) as well as caloric stimulations (Tjernström, 1974) as possible causes of the vestibular reaction

The question is—how does middle ear over- pressure induce vestibular reactions? Fig 3 pre- sents an attempt to give a possible explanation The prerequisite was an overpressure of 60 cm H_2O The figure is designed to assume a pres- sure transmission from the middle ear to the inner ear by way of the oval and the round windows Evidence for such a pressure trans- mission has been demonstrated in our clinic in animal experiments As the inner ear is filled with incompressible fluid, the pressure in the inner ear and that in the middle ear will be roughly equal However, this is possible only if the cochlear aqueduct is of poor patency on exposure to sudden pressure changes in the inner ear There is histopathological as well as experimental evidence that the patency of the cochlear aqueduct is poor, at least on exposure

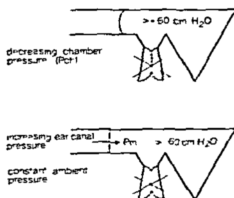


Fig. 1. Middle ear models. For details see text.

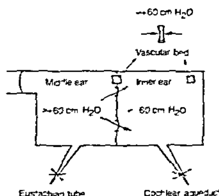
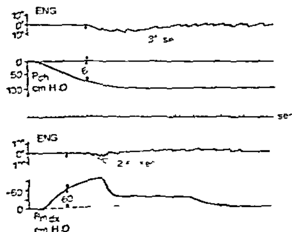


Fig. 3. Schematic model of middle and inner ear ... dotted squares represent the vascular bed. For de... see text.

to sudden pressure changes (Karlefors, 1924, Waltner, 1948, Ritter & Lawrence, 1965).

The pressure required to induce a vestibular reaction was found to be 60 cm H_2O and as the capillary pressure is less than 50 cm H_2O, the presumed inner ear overpressure might cause circulatory insufficiency. One might also imagine another mechanism, namely that the pressure affects blood vessel connections between the middle and the inner ear.

In another series of experiments changes in volume of the middle ear mucosa induced by ...ges in the ambient pressure were studied ...son et al., 1976). Fig. 4 shows a middle ... with a perforated eardrum. The middle ... cavity is in communication with a constant atmospheric pressure via a catheter and a rubber cuff applied into the external ear canal. This means that the absolute pressure in the mid... ear will be constant and not affected by press... changes in the chamber provided that ... Eustachian tube is closed. When the cham... pressure was increased, a flow of air out of ... middle ear was recorded by means of a fl... meter. This flow of air was caused by an ... crease in the volume of the middle ear muco... ΔV_{muc}. This increase in volume was due to ... *ling of blood of the vessels, i.e. a congestion* ... the mucosa caused by the increased press... outside the body. As seen from Fig. 5, a red... tion of chamber pressure reduced the volu... of the middle ear mucosa, a decongestion, whi... could be recorded as a flow of air into the mid... ear. The degree of volume variation was calc... *lated by integration of the airflow.* When t... ambient pressure was reduced, the Eustachi... tube was forced open by the relative overpr... sure in the middle ear. In these experiments ... was also seen that the forcing pressure level w...



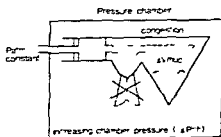


Fig. 4. ΔV_{muc} indicates the volume increase of the mucosa lining the middle ear space and induced by i... creased ambient (chamber) pressure. For details see te...

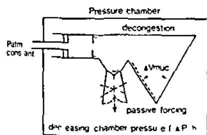


Fig 5 ΔV_{muc} indicates the volume decrease of the mucosa lining the middle ear space and induced by decreased ambient (chamber) pressure For details see text

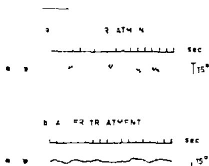


Fig 7 ENG recordings of nystagmus immediately before and after the underpressure exposure

lower when the decongestion of the mucosa was more pronounced

These experiments have shown that it is possible to induce congestion and decongestion of the vascular bed in the middle ear, provided that the middle ear is not in communication with the changing ambient pressure In view of these results it might be possible to induce variations in volume of the vascular bed in any closed rigid cavity of the body What, then, about the vascular bed of the labyrinth?

As mentioned above, there seems to be histopathological as well as experimental evidence that the patency of the cochlear aqueduct is poor when exposed to sudden pressure changes Then the remaining communications out of the bony labyrinth are the endolymphatic duct and the blood vessels Numerous studies on animals and humans seem to give evidence that endolymphatic hydrops might be due to a partial obstruction of the endolymphatic duct (e g Lundquist, 1965 Kimura & Schuknecht 1965, Kimura 1967 Altmann & Zechner, 1968, Clemis & Valvassori, 1968) Endolymphatic hydrops is today considered synonymous with Menieres disease, and thus it might be assumed that an acute attack of Menieres disease might be due to a temporarily total obstruction of the endolymphatic duct Thus the condition of the inner ear of Menieres disease during an acute attack might represent a closed cavity, i e there is no communication with other parts of the body As in the middle ear experiments in which decongestion and passive forcing of the Eustachian tube was seen to occur due to ambient

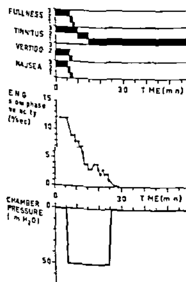


Fig 6 Schematic presentation of subjective and objective symptoms before during and after underpressure exposure Objective symptoms—nystagmus—given as slow phase velocity The figures *1* *2* and *3* indicate the severity of subjective symtoms.

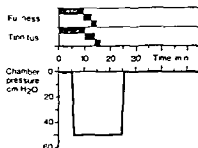


Fig 8 Subjective and objective symptoms before during and after the underpressure exposure

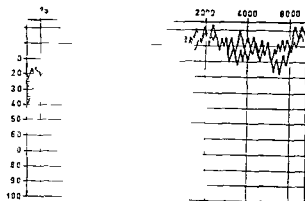


Fig 9 Békésy aud[illegible] immediately before and after the exposure to underpressure Dotted area shows the gain in hearing

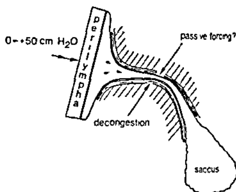


Fig 11 Schematic presentation of the theory mechanism by means of which underpressure e[illegible] might relieve acute symptoms of Meniere's disea[illegible] theory is based on a presumed pressure transm[illegible] from the middle ear to the inner ear, causing a [illegible]gestion of the vascular bed of the inner ear and a p[illegible] passive forcing of the endolymphatic duct

pressure reduction, it might conceivably be possible to affect also the inner ear, inducing a decongestion and a passive forcing of the endolymphatic duct With this in mind it was found of interest to try to find out whether an ambient pressure reduction might affect acute symptoms of Meniere's disease

Patients with acute symptoms of Meniere's disease were thus enclosed in a pressure chamber and exposed to decreasing ambient pressure During the pressure changes the patients were told not to equilibrate the middle ears actively (Ingelstedt et al, 1976)

Fig 6 demonstrates the course of events of the experiments The upper diagram shows the subjective symptoms of the patient before, during and after the exposure to the underpressure

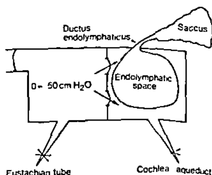


Fig 10 Schematic presentation of middle and inner ear and the endolymphatic duct and sac A relative over pressure (0→ +50 cm H_2O) is induced in the middle ear by ambient pressure reduction, using a pressure chamber For details see text

The diagram in the middle shows the int[illegible] of the spontaneous nystagmus recorded b[illegible] during and after the pressure change an[illegible] lower diagram shows the magnitude an[illegible] duration of pressure change in the cha[illegible] Fig 7 shows the recordings of eye-mover[illegible] immediately before and after the pressur[illegible] duction

About 25% of Meniere's disease patien[illegible] not have vestibular symptoms and ther[illegible] such cases were also included in the experim[illegible] (Densert et al, 1975) The upper diagra[illegible] Fig 8 shows the subjective symptoms and[illegible] lower diagram shows the magnitude and d[illegible] tion of pressure changes Fig 9 demonst[illegible] the hearing improvement evaluated by mea[illegible] a Bekésy hearing test

Thus, it is quite obvious that some case[illegible] Meniere's disease with acute symptoms do[illegible] prove when exposed to ambient pressure re[illegible] tion How might this be explained?

A reduced pressure outside the body cre[illegible] a relative overpressure in the middle ear w[illegible] is indicated in Fig 10 by a pressure increas[illegible] up to +50 cm H_2O The magnitude of the e[illegible] pressure being dependent upon when the E[illegible] tachian tube is forced open passively A [illegible] sumed pressure transmission from the mid[illegible] ear to the inner ear might be expected to ind[illegible] a decongestion of the vascular bed of the in[illegible]

…, mainly the venous system, like the decon…tion seen to occur in the middle ear (cf Fig … Furthermore, like the passive opening of the …stachian tube, the suddenly increased inner … pressure might cause passive forcing of a …nporarily obstructed endolymphatic duct, fa…itated by the decongestion (Fig 11) This will …amatically reduce the endolymphatic hydrops, …ereby relieving subjective as well as objective …ms of the acute attack. *The prerequisite is, …wever, a poor patency of the cochlear aqueduct … exposure to sudden pressure changes in the …ner ear.* Opinions about the patency of the …chlear aqueduct differ widely (Karlefors, 1924, …rlman & Lindsay, 1939; Waltner, 1948, Allen, …)64, Anson et al, 1964, 1965; Holden & …chuknecht, 1968; Palva & Dammert 1969) Is …possible that the degree of patency can differ …om one subject to another? Such an assump…on might explain why some patients with acute …ymptoms of Meniere's disease do not improve …hen exposed to ambient pressure reductions

So far, 46 patients with acute attacks have …een treated, and in about 60% a positive ef…ect has been seen This report is only pre…iminary, but the results are promising and en…ouraging to go on with the experiments

ZUSAMMENFASSUNG

An normalen Versuchspersonen konnte aufgezeigt werden, daß geringfügige Veranderungen des umgebenden Luftdruckes, die im Mittelohr einen Überdruck hervorrufen, vestibulare Reaktionen auslosen können Bei Patienten mit akuten Ménière-Anfällen konnte mit Hilfe des umgebenden Luftdruckes derselben Großenordnung eine Symptomerleichterung erzielt werden

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PATHOPHYSIOLOGY OF THE PARANASAL SINUSES

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Abstract The maxillary ostium is narrower when the subject is recumbent than when sitting The oxygen content in the sinus is related to the patency of the ostium and to some extent to its size A 90 % gas exchange in the sinus normally requires only 5 minutes The exchange is faster during nasal than during oral breathing The mucosa of the maxillary sinus has a relatively high blood flow Oxygen absorption by the mucosa is normally perfusion limited A considerable part of the absorbed oxygen is utilized directly by the mucosa and not taken up by the blood flow

Nobody knows why we have paranasal sinuses, but several hypotheses have been published concerning the function of these sinuses, though none has given any real solution to the problem Even though the physiology of the sinuses is not known many processes involved in their pathophysiology have been investigated There are principally four different factors involved in the pathophysiology of the sinuses studied so far the mucociliary transport the patency of the ostia the gas exchange and the mucosal blood flow

Mucociliary transport

The mucociliary transport in the sinuses has been thoroughly investigated by Messerklinger (1966) and Toremalm et al (1975) and the results in this area are presented by Toremalm in another paper

Patency of the maxillary ostium

It appears that obstruction of the ostia is probably the most important pathophysiological factor in the development of sinusitis By simu neous recordings of the antral and nasal pr sures during breathing sniffing and blowing is possible to make a qualitative separation patent, partially patent, mucus in the osti valve and obstruction also during blowing a sniffing (Drettner, *1965a*) The simultaneous cording also offers an opportunity for quanti tive evaluation, i e to measure the resistance the ostium when it is blocked by mucus or a as a valve

By measuring the resistance to water dur irrigation, a quantitative evaluation is poss when the ostium is obstructed (Drettner 196

However, it is, of interest not only to meas the resistance of the ostium when it is more less obstructed It is perhaps still more imp tant to be able to measure the functional size an ostium which is patent (Aust & Drettr 1974*a*) This can be done by introduction two cannulas into the maxillary sinus One these is used for blowing in air stream (for e ample 2 litres/minute for a few seconds) in the sinus while the other is used for press recording The increase in pressure is indepe ent of the size of the maxillary sinus By co paring the results with a nomogram obtai by using a model with different tube sizes co responding to the maxillary ostium the fu tional size of the maxillary ostium in the i vestigated person can be measured

The size of the maxillary ostium is not co stant but varies with the body posture (Aust Drettner, 1975) When a person is tested in sitting position and then slowly reclined to

This investigation was supported by grants from the Swedish Medical Research Council (Project No 749)

ng position, the ostium size gradually de-ases and especially the last part, from a 30 gree semirecumbent position to the hori-ntal, has a great effect on the ostium These ults are in agreement with those published by nderantz (1969*a*, *b*) concerning the effect of ly posture on nasal patency and patency of Eustachian tube, and illustrate that the re-nbent position may be harmful, especially ing an acute rhinitis episode

The time required for emptying the sinus after has been filled experimentally with contrast dium is also related to the size of the maxil-y ostium This period is longer when the ium is small, and is shorter when the ostium arge (Aust et al, 1976)

ygen exchange in the maxillary sinus

other factor which is of great interest in the hophysiology of the sinus is the gas exchange der normal and pathological conditions The sic knowledge in this field has been achieved studies in dogs performed by Doiteau (1955) have principally investigated the oxygen con it and exchange in the maxillary sinus of in For that purpose a small polarographic O_2-electrode was introduced into the maxil y sinus and the oxygen content measured

The oxygen content is related to the patency the ostium and is about 16 % when the ostium patent, 14 % when it has a valve function and % when the ostium is obstructed (Aust & ettner, 1974*b*) In cases with spontaneously structed otia it was found that the initial ygen content was sometimes as low as a few r cent or even less in purulent sinusitis The ygen content is not only dependent on the tent or non patent ostium, it is also to some ent dependent on the size of the ostium, en the ostium has a functional size of less n 2 5 mm in diameter, the oxygen content in sinus is lower than when the ostium is larger relative insufficiency of the gas exchange ough the ostium giving a low oxygen content the sinus may thus occur This also has an ect on the oxygen absorption by the mucosa, absorption at a steady state being greater when there is a high pO_2 in the sinus t there is a low pO_2

If the oxygen exchange through the is to be investigated separately it is best in model experiments, but the results have been confirmed by experiments in man S experiments are done after filling the mod the sinus with nitrogen and recording the asym totic increase in the oxygen content (Aus & Drettner, 1974*c*) The time required for 90 exchange of the gas in the sinus is inversely cor related to the cross sectional area of the ostium A swelling of the mucosa, for example, by 1 mm in the ostium may thus have a profound influence upon the time required for exchange The nasal respiratory work also affects the gas exchange in the sinus The exchange is thus twice as rapid during nasal breathing than during oral breathing (Aust, 1974) It is normally about 5 minutes during nasal breathing, which is considerably faster than suggested by Proetz (1953) who assumed that the exchange was at least one hour

The normal oxygen exchange through the ostium at a steady state amounts to 0 1 ml/min The oxygen absorption by the mucosa is also 0 1 ml/min in steady state (Aust & Drettner, 1974*d*)

Blood flow of the mucosa in the sinus

In order to get a complete view of the oxygen exchange in the mucosa it is necessary to be able to measure the blood flow of the mucosa in the maxillary sinus This may seem to be a difficult task since no one has so far been able to measure quantitatively the blood flow of any mucosa in the human body But the maxil lary sinus is perhaps the best object for such a study because, as it is situated in a cavity with rigid walls, the volume of the sinus can be measured, the area of the mucosa calculated, and the sinus has only one opening to the outside, which can be blocked experimentally Furthermore, it was found that when the ostium is blocked experimentally, there are always pulse waves at a pressure recording from the (Drettner & Aust, 1974) When the

veins in the neck are compressed, the rise in this recording is more pronounced during bilateral than during homolateral compression of the veins When the carotid artery is compressed, there is a downward slope and a disappearing of the pulse waves

Theoretically there is, however, still a possibility that the rise during jugular vein compression may be due principally to an increase in the venous pressure We therefore carried out a series of experiments in five normal sinuses with introduction of a small quantity of a radioactive gas [133]Xe after tamponade of the ostium (Aust et al , 1977) The disappearance rate was recorded by a gamma detector An initial phase when there was no decrease in the xenon counting was probably due to the fact that xenon has a high density and initially lay in the posterior part of the sinus, when the subject was lying down After a while it was mixed in the whole sinus and consequently closer to the gamma detector The disappearance of the blood is thus initially compensated by this process An experiment in a cadaver showed no elimination after the initial period In the living subjects there was a relatively good correspondence between the results obtained with plethysmography . and after the introduction of xenon and obtained by disappearance rate of the gas shows that the blood flow in the mucosa of the maxillary sinus can be measured by the described plethysmography However, this method still contains several errors, the most serious being the difficulty of achieving a complete and uniform arrest of the venous blood flow in the neck

The blood flow in different tissues of the body is usually calculated in ml per 100 g tissue and minute The problem when the values from the maxillary mucosa are expressed in this way is that the thickness of the mucosa is not known In the literature it is said to be 0 125 mm (Loring & Tenney, 1955) If this value is used the blood flow will be about 100 ml per 100 g tissue and minute which is about the same value as reported for the nasal mucosa by Änggård (1974) and Malm (1974) independent of each other Such a blood flow is considerably gre than in muscles, it is double that of the br similar to that in the liver, while it is less the blood flow in the kidney and the lungs

Oxygen absorption in relation to the blood fl

The fact that both the blood flow and the gen absorption in the maxillary sinus can measured, provides an opportunity to ana the kind of restriction of the oxygen absorpt i e is it diffusion- or perfusion-limited? It provides a possibility to separate the ox absorption into fractions—absorbed by blood or directly utilized by the mucosa culation of the oxygen quantity which m mally can be absorbed by the blood flow in mucosa shows that this absorption is perfus limited and no diffusion limitation occurs d ing normal conditions However, this oxy which maximally can be absorbed is less th the measured value of the absorption, and thus also a direct consumption of oxygen the mucosa It seems likely that this latter o gen fraction is utilized, for example, for ciliary activity

When the results of this present study compared with those reported by Toremalm al (1975) it is obvious that the oxygen conte in the sinus can reach such low values whe the mucociliary activity decreases according Toremalm et al Our results also indicate a d rect utilization of oxygen by the mucosa How ever, the background of these processes has n yet been studied and the oxygen exchange an the blood flow under pathological conditio are projects which will be studied in the ne future

ZUSAMMENFASSUNG

Das Kieferhohlenostium ist in liegender Lage enger a in sitzender Lage Der Sauerstoffgehalt in der Kiefe hohle ist von der Durchlässigkeit und zum gewisse Teil auch von der Große des Ostiums abhängig Ei Austausch von 90 % des Gases in der Kieferhohle daue

fluß Die Sauerstoffabsorption der Schleimhaut ist ise

-ise perfusionsbegrenzt Ein ansehnlicher Teil der absorbierende Sauerstoff, wird direkt von der Schleim- verbraucht und wird nicht vom Blut aufgenommen

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SYMPATHETIC INFLUENCE ON THE NASAL MUCOSA

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Abstract The tone of the resistance vessels determines the capillary flow and the tone of the capacitance vessels —the nasal patency Lower impulse frequencies in the sympathetic nerves affect mainly the capacitance vessels, while higher frequencies affect both types of vessel The existence of both α- and β adrenoreceptors and their distribution offer pharmacological ways to affect mainly one type of the vessels

The blood vessels in a tissue can be divided functionally into resistance vessels, capillaries, and capacitance vessels The resistance vessels control the blood flow, through the capillaries the exchange between blood and tissue takes place, and the capacitance vessels contain nearly all of the blood and thus determine the blood volume or blood content of a tissue Such a basis of division is especially suitable in the nasal mucosa as this both has many arteriove-us anastomoses which can change the blood ow, and is richly supplied with sinusoids, hich are typical capacitance vessels

The degree of mucosal congestion or nasal patency is mainly determined by the tone of the capacitance vessels The tone of the resistance vessels, on the other hand, is of importance for the surface temperature of the nasal mucosa Owing to the differing tasks of the two types of vessel it is reasonable to assume that they can be controlled by nerves and hormones independently of each other In order to investigate if this is true for the sympathetic system the effects of sympathetic nerve stimulation and of sympathomimetic drugs on the nasal vessels of the anaesthetized cat were studied

Two methods to determine changes in the resistance and the capacitance vessels, were elaborated (Malm 1973, 1974*a*) To measure the nasal blood flow, the pterygopalatine vein, which drains most of the mucosa of one ca was cannulated and the drops of blood recorded with a flowmeter At the same ti changes of the nasal patency of the same ca were measured by recording pressure change a water-filled balloon positioned in that cavi

Fig 1 shows the results of electrical stim tion of the sympathetic nerves to the nose of cat On the abscissa are the frequencies u and on the ordinates the simultaneously tained changes in the resistance and the pacitance vessels The changes are related to changes at 10 imp/sec The resistance and capacitance changes differ significantly with 0 1 and 5 imp/sec, and this means that capacitance vessels constrict relatively more low frequencies than do the resistance vesse Thus, even when both types of vessel are nervated by the same sympathetic nerves, chan ing the impulse frequency can affect one ty of vessel relatively more than the other Such e fects of stimulation of sympathetic nerves the feline nasal mucosa have also been demon strated with radioactive methods by Änggår & Edwall (1974)

There are two types of sympathetic receptors α-adrenoceptors and β-adrenoceptors When α adrenoceptors are stimulated, blood vessels constrict and when β-adrenoceptors are stimulated, blood vessels dilate

It is generally accepted that α-adrenoceptors exist in nasal blood vessels both in resistance vessels and in capacitance vessels The existence of β-adrenoceptors in the nasal vessels is, however, more debated Hall & Jackson (1968) could not demonstrate β-adrenoceptors in the nasal blood vessels of the dog They used a

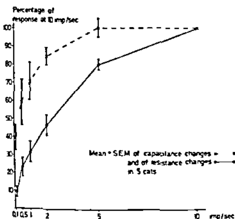


Fig 1 Mean changes ±S E M of blood flow resistance (——) and of capacitance changes (--) in per cent of the changes at stimulation with 10 imp/sec in 5 cats

method, however, which determines changes in capacitance vessels only With the present author's method of measuring changes in the resistance vessels, β adrenoceptors were demonstrated in the feline nasal mucosa (Malm, 1974*b*) Isoprenaline, which is a pure β receptor stimulating agent, was given to cats intra arterially near the nose This resulted in a vasodilatation, which in Fig 2 is represented as a decrease in blood flow resistance The slope of the regression line through the mean values after different doses of isoprenaline is, however, not sufficient to prove the existence of β adrenoceptors Therefore the β-receptor blocking agent propranolol was administered Subsequently, bigger doses of isoprenaline were necessary to

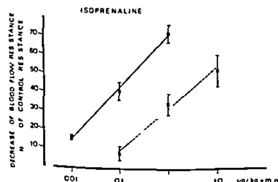


Fig 2 Mean changes +S E M of nasal blood flow resistance in per cent of control after i a infusions of isoprenaline before (——) and after (--) propranolol 1–3 mg kg i v in 6 cats.

evoke vasodilatation, as seen from the dashed line The slope of the regression lines and the distance between them clearly indicate that β-adrenoceptors exist in resistance vessels of the cat's nasal mucosa β-adrenoceptors could not, however, be significantly demonstrated in the capacitance vessels, which may indicate no or only a few β adrenoceptors in these vessels

If such an uneven distribution of β adrenoceptors exists in the nasal vessels, drugs, acting mainly on α adrenoceptors, may have a strong constricting effect both on resistance and on capacitance vessels, while other drugs, for instance ephedrine, with a rather good effect on both α and β-adrenoceptors, may strongly constrict the capacitance vessels but less strongly constrict the resistance A strong constriction of the resistance vessels, which is the same as decreased blood flow through the capillaries, may be unsuitable in disease conditions Knowledge of such matters is of importance, especially as nearly all nose drops are pure α receptor stimulating drugs

ZUSAMMENFASSUNG

Der Tonus der Widerstandsgefasse bestimmt den kapillaren Blutstrom und der Tonus der Kapazitanzgefasse die Durchlassigkeit der Nase Geringe Impulsfrequenzen in den sympatischen Nerven beeinflussen hauptsächlich die Kapazitanzgefässe, während eine Steigerung beide Arten von Gefassen beeinflusst. Die Existenz von sowohl α als β Adrenoceptoren und ihre Verteilung geben eine pharmakologische Voraussetzung fur den Einfluss auf hauptsächlich eine der beiden Gefassarten

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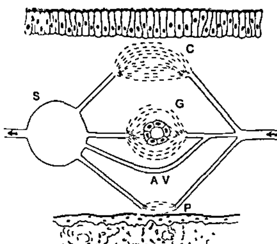


Fig 1 The vascular arrangement in the nasal mucosa *A-V* Arteriovenous shunt vessel *C* = Subepithelial capillaries *G* = Periglandular capillaries *P* – Perichondral or periosteal capillaries *S* = Venous sinusoids

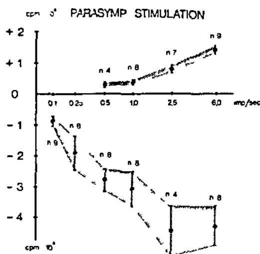


Fig 2 Influence of parasympathetic and sympathetic nerve stimulation on the gross pulse rate of ^{131}I-albumin measured over the right nasal cavity Mean, S E M and number of observations are shown

with the results obtained in similar experiments where the effects of graded stimulation of the sympathetic fibres were studied (Änggård et al, 1974), the maximal effects following sympathetic nerve stimulation were 3–4 times greater than the effects of parasympathetic nerve activation (Fig 2) Furthermore at discharge rates of 0 5 imp/sec the parasympathetic effects on local blood content were very small compared with the sympathetic effects

The effects of parasympathetic nerve activation on the exchange and capacitance vessels are illustrated in Fig 3 Stimulation with frequencies from 0 5 imp/sec consistently resulted

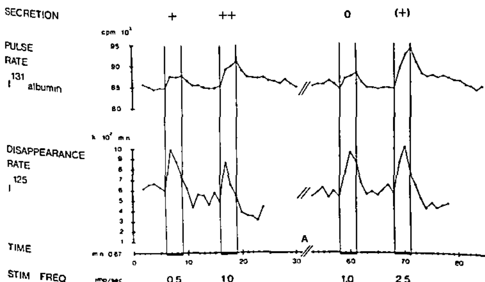


Fig 3 Influence of parasympathetic nerve stimulation on tracer disappearance rate of ^{125}I and the gross pulse rate of ^{131}I albumin measured over the right nasal cavity The secretory response is indicated above the curves None, 0, slight (+), noticeable, + , obvious, + + . Cat 2 4 kg (A) Intra arterial infusion of atropine 1 mg/kg

in an increase in tracer disappearance rate indicating an increased tissue-blood exchange Simultaneously increases in secretion and local blood content were observed To investigate whether or not the effects were due to activation of cholinergic fibres a high dose (1 mg/kg) of atropine was given This blocked the secretory response to parasympathetic stimulation However, atropine did not block the increases in tracer disappearance rate or local blood content

The present results thus suggest that the vascular and secretory responses in the nasal mucosa are activated simultaneously at parasympathetic nerve activation The secretory response (but not the vascular events) might be blocked by atropine Hence, the postganglionic parasympathetic mediator of nasal secretion is cholinergic, whereas the vasodilatation appears to be due to a different mechanism, which is not sensitive to atropine

Furthermore, it is probable that under physiological conditions autonomic nerve fibres have a discharge rate around 1–2 imp/sec At this rate of parasympathetic discharge to the nasal mucosa, major changes would occur only in secretion and capillary exchange function The local blood content would increase only to a minor degree and nasal patency would therefore not be appreciably altered

ZUSAMMENFASSUNG

Die Wirkung der selektiven parasympathischen Nervenaktivierung auf die sekretorische Reaktion und auf d vaskulare Austausch und Fassungsvermogen der Nase schleimhaut wurde an der Katze untersucht und d Resultate werden hier diskutiert

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QUANTITATIVE STUDIES OF GAS ABSORPTION FROM THE NORMAL MIDDLE EAR

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Abstract Gas absorption in the normal ear was determined quantitatively The composition of the gas mixture is discussed and compared with other biological gas pockets.

There has been a good deal of discussion about the gas physiology in the middle ear and some questions have been put forward How does the absorption of gases take place and what are the characteristics of this process? How large an amount is absorbed and what is the resulting underpressure? Is there any difference between large and small mastoid cell systems? What is the composition of the gas mixture in the middle ear?

This is basic physiology but the questions—or rather their answers—might have clinical implications

According to Herman Rahn (1963), who is physiologist in Buffalo, we only have to deal with three types of biological "gas pockets" (Fig 1) The normal ear is regarded as an "open non ventilated" or "intermittently ventilated gas pocket" which means that both oxygen and nitrogen are absorbed but carbon dioxide is in constant equilibrium with the surrounding tissues

A certain amount of work has been done on gas absorption in cases with drum perforations, mainly by Riu et al (1966) in Toulon We were however, interested in what happened in the normal ear behind an intact tympanic membrane and we have used a technique that does not disturb the normal physiology When the Eustachian tube is kept closed, this will—as everyone knows—create an underpressure in the ear because of the continuous absorption of oxygen and nitrogen (Fig 2) This causes the tympanic membrane to retract slowly and —as the middle ear is not a rigid box—the mucosa to increase its volume in consequence The change in the volume of the mucosa counteracts the inward movement of the drum so both these volumes must be taken into account when we calculate the absorbed volume Furthermore, there is also a change in the *mass* of the enclosed gas, ΔV_m, and that is also a part of the absorbed volume Therefore, to get the correct value we have to add these three volumes

$$V_{diff} \quad \Delta V_{tm}+\Delta V_{muc}+\Delta V_m$$

It is also clear that the input to the ear through the Eustachian tube at equilibration must be the same as the output from the ear by absorption, provided that there are no other ways that air can enter or leave That raises the question whether diffusion of the respiratory gases can take place through the intact tympanic membrane? Based upon differences in partial pressures, nitrogen can pass from the ear to the ear canal and oxygen from the ear canal to the ear (Fig 3) But the volumes are so small—as we have demonstrated on fresh tympanic membrane preparations—that they can be disregarded, compared with the 24-hour volume passing the Eustachian tube

Of the three components above we only have to *record* the volume displacement of the tympanic membrane ΔV The volume c

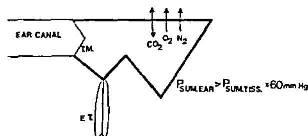


Fig 1

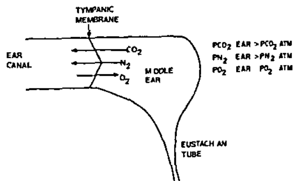


Fig 3

mucosa per cm H_2O underpressure, ΔV_{muc}, we know from earlier experiments by Ingelstedt et al and the change in the mass of the gas, ΔV_m, can be calculated when we know the volume of the middle ear and mastoid cells

When the tympanic membrane springs back from a retracted position to its normal position as the examined person equilibrates by swallowing after having kept the Eustachian tube closed for 5–10 min, we get a flow in the ear canal and this can be recorded by an ultrasensitive flow meter connected to the ear canal with a cuff system (Fig 4) This volume displacement of the drum is the same as the inward displacement during the foregoing period of increasing underpressure The *flow* is integrated to a *volume*, ΔV_{tm} Further, one can calculate the resulting underpressure in the ear and thereby also obtain the change in the volume of the mucosa

With this technique we have determined the absorbed volume in 5 normal persons with repeated recordings two or three times, getting the same values (Fig 5) The mean value per hour is 33 microlitres Provided that the absorption conditions are the same during 24 hours one can also get 24-hour values by extrapolating for that time The values range between 0 7 and 1 1 ml for 24 hours But, as we spend about 8 out of 24 hours in a recumbent position and as we know that the volume of the mucosa increases somewhat because of the increased venous pressure in this position the diffusion might be different during the period of sleep It would be interesting to study this but unfortunately an absolutely perfect equilibration capacity is a prerequisite for the recordings and we know that the tubal function is somewhat impaired in the recumbent position This makes recordings very difficult if not impossible

There seems to be no correlation between the absorbed volume and the volume of the middle ear and mastoid cells Presumably the bulk of the oxygen and nitrogen is absorbed in the middle ear cavity itself This has a more vascularized mucosa and about the same volume in all individuals This assumption is supported by experiments with radioxenon, injected in the mastoid cells and middle ear in a group of per

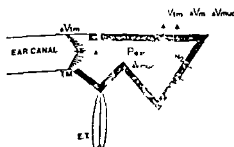


Fig 2

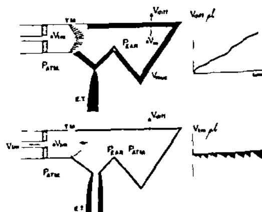


Fig 4

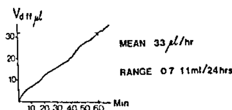


Fig 5

sons with a tracheostoma and differing known ear system volumes Xenon is cleared nearly 100% through the lungs and we could not find any differences in activity in the expired air collected from the tracheostoma or in the disappearance rate of the gas from the ear system

The values of gas absorption determined by this indirect technique are well in agreement with those of others, using other techniques and methods (Table I) Riu and co-workers in Toulon found 0 8 ml per 24 hrs Ingelstedt & Jonson punctured the mastoid cells and calculated the absorbed volume from the pressure drop, and found 1–2 ml If we go further back, van Dishoeck, using his pneumophone method recorded an underpressure in the ear of 5–8 cm water per hr and this corresponds to about 0 5–1 0 ml Thus, even if there are some possible errors such as that we do not know if the absorption is altered during the night, it seems justified to state that the volume of air absorbed from the ear is about 1 ml for 24 hrs[1]

The resulting underpressure can easily be calculated when we know the absorbed volume and the volume of the air filled space of the middle ear and mastoid cells It varies according to the size of the middle ear system A large middle ear system gives a small underpressure and vice versa But an average of about −5 cm water for the first hour seems reasonable

A most interesting point—and lately a somewhat controversial one—is the composition of the air in the middle ear (Table II) Melvill Jones (1958) found 84% nitrogen, 9% oxygen and 7% carbon dioxide Riu and co-workers found very similar values when using a gas chromatograph method If we compare these values with those found in experimental subcutaneous gas pockets in rats in steady state (Fig 6), they are surprisingly similar Is it therefore possible that the middle ear system has a gas composition that differs from inspired as well as expired air and corresponds in that respect to other biological gas pockets? There is some evidence for this *Firstly*, the mean volume of the middle ear is 6 ml (6 000 microlitres) and if about 1 µl passes up the Eustachian tube at equilibration (we swallow once every minute or every second minute) it does not seem likely that the small volume of 1 µl could change the gas composition of 6 000 µl *Secondly*, Riu et al did find the same values in cases with a blocked Eustachian tube as well as in subjects with normal functioning tubes Some of their subjects even had a transudate in the ear *Thirdly*, Ingelstedt et al determined the oxygen and carbon dioxide tension in transudate from the ear and their values are nearly the same (Table II)

Table I *Gas absorption from the middle ear in 24 hrs*

Riu et al (1966)	0 8 ml
Ingelstedt & Jonson (1967)	1 2 ml
Elner et al (1971)	0 7 1 1 ml
[van Dishoeck (1941) 5 → 8 cm H_2O hr corresponding to ~0 5–1 ml]	

Table II *Gas composition in the middle ear (1 and 2), in elastic, subcutaneous gas pockets (3), and in transudate from the middle ear (4)*

	N_2	O_2	CO_2
1 Melvill Jones (1958)	84%	9%	7%
2 Riu et al (1966)	85%	9 5%	5 5%
3 Rahn & Canfield (1955)	88 3%	5 7%	6%
4 Ingelstedt et al (1975)	—	5 5%	8%

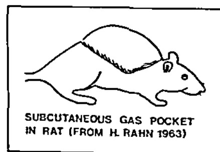


Fig 6

There are of course many sources of error and the techniques being used are both difficult and delicate The values of oxygen and nitrogen might be discussed, but according to the physiologists, carbon dioxide is diffusing so fast that a state of equilibrium with the surrounding tissues is reached very rapidly in *every* closed air-filled cavity in the body We have about 40–45 mmHg of carbon dioxide in our tissues and there seems to be good reason to assume that there should be about the same values in the ear However, Sadé (1976) has recently found considerably lower concentrations of carbon dioxide in normal ears

CONCLUSION

The volume absorbed from the ear is about 1 ml per 24 hrs The underpressure developing is about −5 cm water for the first hour The ear has a steady state gas composition similar to other biological gas pockets (?) There is no *ventilation* of the ear in the proper sense of the word via the Eustachian tube, but a mere pressure regulation

ZUSAMMENFASSUNG

Das Volumen von dem Ohr absorbiert ist ungefähr 1 ml in 24 Stunden Der erstandene Unterdruck der ersten Stunde ist ungefähr −5 cm Wasser Das Gas des Ohres hat dieselbe steady state Zusammensetzung als das derer biologischer Gastaschen(?) Es gibt im Ohr keine Ventilation — im eigentlichen Sinne des Wortes — durch die Ohrtrompete sondern nur eine Regulation des Druckes

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CORRELATION OF TUBAL FUNCTION AND VOLUME OF MASTOID AND MIDDLE EAR SPACE AS RELATED TO OTITIS MEDIA

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Abstract A connection was found between impaired tubal function and a small ear space volume The correlation of these two parameters indicates the degree of mucosal impairment in chronic otitis media

There has always been much discussion about the relation between the size of the mastoid air cell system and chronic otitis media Diamant (1940) believed that the size of the air cell system is genetically determined, and that small air cell systems are a cause of chronic otitis media, while Tumarkin, like Wittmack, claimed that middle ear disease starts very early—even before delivery—and that small cell systems are not the cause but the effect of the infection

The value of testing preoperative tubal function is also much discussed, and we know that many surgeons do not use such tests In our opinion, however, one should study not only the function of the Eustachian tube, such function being only one of the aspects of the impairment of the functional system in chronic otitis media The mucosal lining of the middle ear, the mastoid air cell system, and of the Eustachian tube, is presumably systematically damaged in chronic otitis media, resulting in an impairment of tubal function together with a successive reduction of the air-filled ear spaces as a result Holmquist (1970) and Siedentop (Siedentop et al, 1969) have both studied the correlation between tubal function and the area of the cell system measured planimetrically They found that a correlation exists between large cell systems and good tubal function as well as between small cell systems and impaired tubal function We do not believe that the radiographic area of the cell system is a true mirror of the functioning volume, since parts of it may be obstructed by pathological changes of the mucosa not visible on an X-ray picture We therefore tried to ascertain whether and, if so, to what extent tubal function varies with the volume of the functional air spaces on the one hand and the roentgenographic area on the other

The investigation was done on 69 ears with central perforation following chronic otitis media and on 9 ears with a recent traumatic perforation Only persons whose ears were dry and who exhibited no symptoms or signs of upper respiratory tract infection were accepted Fig 1 shows the equipment used both for tubal function studies and volume measurements It consists of an air-tight glass syringe with a micrometer screw hermetically connected to a pressure transducer The device and the ear form a closed system and by altering the middle ear pressure with the syringe and asking the patient to swallow, one can measure manometrically the function of the Eustachian tube according to the aspiration-deflation method Elner et al (1971) divided their normal material into four groups according to the aspiration-deflation method (Fig 2) Group I perfect aspiration-deflation capacity, group II somewhat impaired aspiration-deflation, group III no aspiration capacity but with deflation capacity, and group IV, no function at all Fig 3 shows a direct comparison

This paper was prepared together with Alf Ivarsson, Engineer

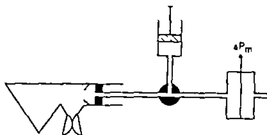


Fig. 1. Equipment for tubal function studies and volume measurements.

of Elner's 102 controls with the 69 cases of chronic otitis media in my investigation. There are differences which could be expected with the lower percentage of tubal function group I in my investigation and the higher percentage in function groups III and IV. If one were to combine groups I and II, i.e. patients with ability to aspirate and called the positive aspiration group, we would see that 70% of the patients with chronic otitis media had a positive aspiration capacity, compared with 93% in Elner's material.

Table I shows a comparison between this investigation and other tubal function studies in chronic otitis media. There is a considerable difference between Sharp's 7.5% (1970) with a positive aspiration capacity and my 70%.

The differences probably depend mostly on the selection of the patients. In our material the ears had been quite dry for at least one month, besides which the patients showed no signs of upper respiratory infection and the ears no signs

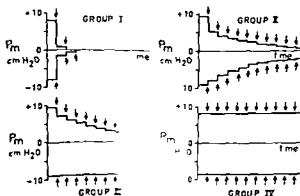


Fig. 2. Classification o[...] ity to equalize an int[...] H₂O and the extratymp[...] of swallowing. [...]n according to abil[...] pressure of +10 cm [...] Arrows denote acts

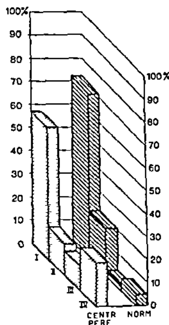


Fig. 3. Comparison of the tubal function in healthy e[...] vs. ears with chronic otitis media.

of cholesteatoma. The differences can also d[...] pend on other factors, for example if a h[...] negative pressure is applied in the middle e[...] for some minutes one gets a sucking effect [...] the mucosal lining, with consequent impair[...] tubal function. In the upper part of Fig. 4 t[...] principle for determining the volume is show[...] There is a closed system with an unknown vo[...] ume, V_m. A known volumetric change, Δ[...] causes a pressure change, ΔP_m, which can [...] measured. The ambient pressure, P_m, is the actu[...] atmospheric pressure, minus the pressure [...] water vapour which is 47 mmHg, and from the [...] known variables one can determine the V_m wi[...] Boyle's Law.

In the lower part of the figure one can s[...] the ear and the measuring device hermeticall[...] connected with a polyethylene catheter and [...] cuff. If we alter the pressure to −5 cm H₂[...] and read the volume change of the micromet[...] screw necessary for this pressure change w[...] can calculate the total volume of the clo[...] system. The volume of the measuring device [...] known and the volume between the level of t[...] tympanic membrane and the cuff has been c[...] culated to be 0.3 ml. Subtraction of these tw[...]

Table I *Comparison between different investigations concerning aspiration capacity, number of swallows and test pressures (a) ears with normal mucosa out of a larger material (b) ears with central perforation and intact ossicles out of a larger material*

Invest	No	Pos asp (%)	No of swallows	Test pressure (cm H_2O)
Miller (1965)[a]	54	59	1–36	25
Flisberg (1966)	96	42	Not given	2–40
Miller & Bilodeau (1967)	91	63	Not given	2 5–25
Siedentop et al (1969)[a]	144	44	1–>6	25
Holmquist (1968)	217	48	Not given	20
Ekvall (1970)[b]	21	38	≥10	2–50
Sharp (1970)	40	7 5	1–30	10–30
Andreasson et al (1975)	69	70	1–10	10

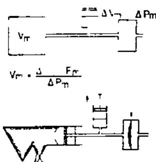


Fig 4 The principle of volume determination (*top*) The sources of error indicated as shaded areas (*bottom*)

volumes from the total gives the volume of the air filled ear spaces

There are three sources of error, indicated as shaded areas in the lower part of the figure Firstly, the membrane deviation which in this set up is negligible Secondly, the cuff can slip if not placed correctly Careful positioning of the cuff in the bony part of the outer ear canal practically precludes sliding Thirdly, any change in the pressure acts on the vessels of the mucosa lining the cavities causing volumetric errors, making correction for this mucosal factor necessary This mucosal factor has been calculated to be 1 μl/cm H_2O and constant within a pres sure range of −15 to −15 cm H_2O (Andreasson et al, 1975) The projection used of the planimetric measurement is called Runstrom's Projection No 2 The borders of the cell system are outlined on paper and the area measured with a planimeter

Fig 5 shows a direct comparison between the area and the volume The linear regression shows a statistically significant difference in the area/volume relation in the cases of chronic otitis media compared with the cases of traumatic perforation This means that one might expect a smaller volume in a case of chronic otitis media than in a case of traumatic perforation Parts of the cell system are probably shut off by pathological changes The planimetrically measured area shows only the size of the mastoid air cell system, whereas the volume method shows the really functional volume

In Fig 6 the tubal function is correlated with the size of the mastoid air cell system measured both as an area and as a volume The mean area in the cases of traumatic perforation was significantly larger than that in the positive aspiration group On the other hand, no statistically significant difference was found between the positive and the negative aspiration group The mean volume was significantly larger in the cases of traumatic perforation than that in the positive aspiration group, which in turn was significantly larger than in the negative aspiration group Thus volume varies more closely than area with tubal function Combined testing of tubal function and volume permits an estimation of the mucosal damage in the whole function unit—

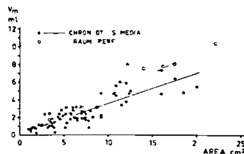


Fig 5 Relation between the area and the volume results.

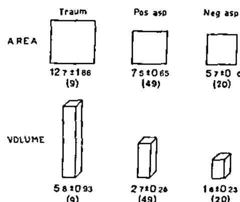


Fig 6 Mean area and mean volume in cases of traumatic perforation and chronic otitis media (positive and negative aspiration capacity)

the middle ear, the air cell system and the Eustachian tube

✓The mastoid air cell system is fully developed at 15 years of age, according to Diamant (1940), and therefore I selected patients in whom the onset of the disease according to the medical records occurred after that age (Fig 7) I then plotted the duration of the disease relative to the measured functional volume and the area The volume decreases with the duration of the disease, but no such relation was found between area and duration These observations show that longstanding chronic otitis media with recurrent discharge reduces the functional volume by successively shutting off parts of the cell system This investigation furthermore shows the necessity of early surgery ✓

We are testing all patients with chronic otitis media who are to be operated on We are testing tubal function and measuring functional

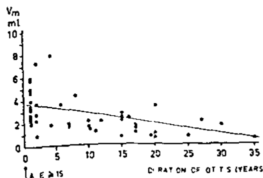


Fig 7 Relation between the duration of the chronic otitis media and the volume results

volume This is a simple process, it takes only 15 minutes All patients are operated on, regardless of the function, but we are of the opinion that cases with poor tubal function and small cell system should preferably be operated upon by a highly qualified surgeon

In conclusion, we feel that determination of tubal function and the functional volume is a better indicator of the severity of the mucosal damage than is tubal function alone or combined with roentgenography Whether the results of our preoperative examinations are correlated with the results of operation cannot yet be decided, but we are trying to clear up this point

ZUSAMMENFASSUNG

Ein Zusammenhang zwischen herabgesetzter Tubenfunktion und geringem Rauminhalt des Mittelohres konnte aufgewiesen werden Die Korrelation zwischen diesen beiden Parametern zeigt das Ausmaß der Schleimhautschädigung bei chronischer Mittelohrentzündung an

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for example evaluation of drug effects However, there are some basic problems which must be investigated and solved before these methods can be used regarding the mucociliary function

Firstly, the temperature factor A temperature rise from 20°C to 40°C means an increase in mucociliary wave movements from about 420 to 1 000 waves per minute The optimal function takes place at body temperature Therefore exposure experiments regarding the mucociliary activity ought to be done at body temperature rather than at room temperature The frequency/temperature relationship is not strictly logarithmic or linear (Fig 4, above) The regression line levels out slightly at temperatures at and above body temperature (Mercke et al , 1974*b*)

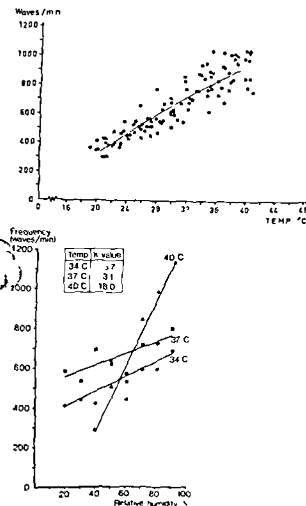


Fig 4 The relationship between mucociliary activity and temperature (*above*) and between different degrees of air humidity and mucociliary activity (*below*)

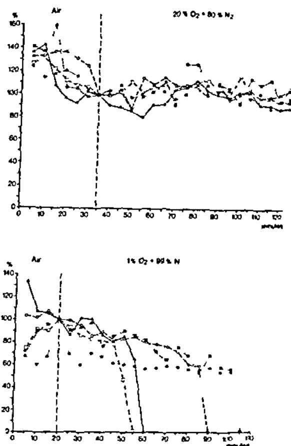


Fig 5 The mucociliary activity in rabbit trachea in a 20 % and 1 % oxygen/nitrogen mixture Temperature, 37°C Humidity, >90 %. The mucociliary activity is indicated in percent of the initial value

Secondly, the relative humidity of the surrounding air is of mandatory importance for the cilia When reduced from 90 % to 50 % at 37°C successive reduction in function is observed From the regression lines, shown below in Fig 4, it is obvious that a reduced humidity is more dangerous at high temperatures (Mercke, 1975)

Thirdly, the mucociliary activity is an aerobic process *In vitro* the ciliated cells are consuming oxygen from the surrounding air via the secretion layer The activity is maximal in an atmosphere of ordinary air (20 % oxygen) but even 1 % oxygen is enough to maintain the activity for a short period of time Pure oxygen on the other hand, has no toxic effect During oxygen therapy, for example, the dryness of the gas is more dangerous than the gas itself A rapid

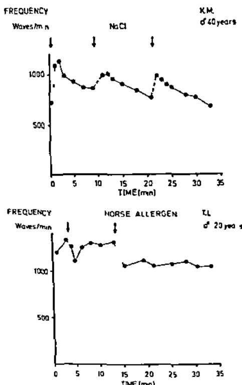


Fig 6 Mucociliary wave movements after application of NaCl solution and horse allergen respectively Tempera ture 37 C Relative humidity >90 %

ciliestasis can be observed in an atmosphere where oxygen has been replaced by pure nitrogen or carbon dioxide (Fig 5) (Reimer & Toremalm, to be published)

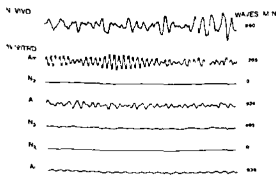


Fig 7 Mucociliary activity recorded *in vivo* during a Luc Caldwell operation for chronic maxillar sinusitis (*above*) *In vitro* recordings on a specimen from the same area, exposed to air and nitrogen respectively at 37 C and a relative humidity 90 %

Finally, I will present very briefly some actual research projects regarding the effects of allergens, bacteria and antibiotics on the extracellularly recorded mucociliary function (Reimer et al , 1976)

1 Firstly, when the mucous membrane was exposed to a solution of sodium chloride the activity was *increased* due to reduced viscosity (Fig 6, above) However, when the cilia of a horse-sensitive mucosa were exposed to a solution with horse allergens, the activity was *reduced* (Fig 6, below)

2 How do the cilia react in an enclosed infected cavity such as a sinus or a middle ear? Cilia seem to tolerate some pathogenic bacteria very well This can be seen during *in vivo* recordings from patients with chronic maxillar sinusitis during Luc Caldwell operations Recordings *in vivo* and *in vitro* after removal of a specimen from the posterior wall are illustrated in Fig 7 It can also be shown experimentally when a substrate with an embedded trachea is inoculated with bacteria

3 Thirdly, do antibiotics retard or stop the ciliary activity in sinuses or middle ear cavities? In spite of the phylogenetic equality between sensory cells of the inner ear and the cilia we could not find any reduction of the ciliary activity after exposure to gentamicin in a 10 % solution for 3 hours However, comparative studies with penicillin preparations showed a rather rapid ciliestasis even within ½–2 hours These preliminary pharmacotoxic experiments will be continued and extended

After this very brief review, one can readily understand why we find the mucous membranes of the respiratory tract a fascinating world to work in and—if carefully handled—the cilia are very cooperative structures for clinical research

ZUSAMMENFASSUNG

Die mucoziliare Aktivitat der Atemwege wird als experimentelles Modell benutzt fur Expositionsstudien betreffs Luftverschmutzung und pharmakologischer Substanzen Es ist moglich, gleichzeitig mit den extrazellularen mechanischen Zilienbewegungen die intrazellulare elektrische Aktivitat zu messen

for example evaluation of drug effects However, there are some basic problems which must be investigated and solved before these methods can be used regarding the mucociliary function

Firstly, the temperature factor A temperature rise from 20 C to 40 C means an increase in mucociliary wave movements from about 420 to 1 000 waves per minute The optimal function takes place at body temperature Therefore exposure experiments regarding the mucociliary activity ought to be done at body temperature rather than at room temperature The frequency/temperature relationship is not strictly logarithmic or linear (Fig 4, above) The regression line levels out slightly at temperatures at and above body temperature (Mercke et al, 1974*b*)

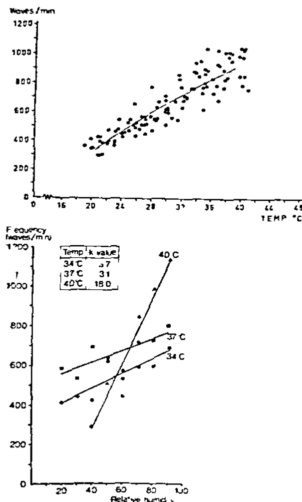


Fig 4 The relationship between mucociliary activity and temperature (*above*) and between different degrees of air humidity and mucociliary activity (*below*)

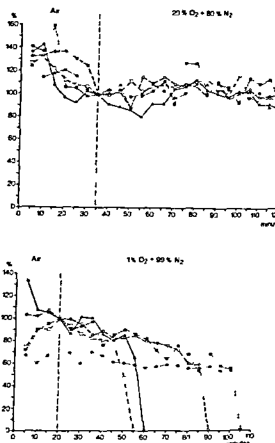


Fig 5 The mucociliary activity in rabbit trachea in 20 % and 1 % oxygen/nitrogen mixture Temperature 37 C. Humidity, >90 %. The mucociliary activity is indicated in percent of the initial value

Secondly, the relative humidity of the surrounding air is of mandatory importance for the cilia When reduced from 90 % to 50 % at 37°C successive reduction in function is observed From the regression lines, shown below in Fig 4, it is obvious that a reduced humidity is more dangerous at high temperatures (Mercke 1975)

Thirdly, the mucociliary activity is an aerobic process *In vitro* the ciliated cells are consuming oxygen from the surrounding air via the secretion layer The activity is maximal in an atmosphere of ordinary air (20 % oxygen) but even 1 % oxygen is enough to maintain the activity for a short period of time Pure oxygen, on the other hand, has no toxic effect During oxygen therapy, for example, the dryness of the gas is more dangerous than the gas itself A rapid

CONCLUSIONS OF THE SYMPOSIUM

S Ingelstedt

To try to draw any conclusions or to sum up this symposium in a few words would be impossible, but all these subjects that we have now discussed have one thing in common—they all belong to the field of clinical physiology We consider it a great privilege to work together with specialists in clinical physiology which is a specialty on its own at the larger hospitals in Sweden

We thank you for letting us present these current investigations to the eminent members of the Collegium, and we hope the discussion will continue in a more informal way during the following days when we have the pleasure of being together

Finally, we in the symposium would like to thank our President, Professor Carl Axel Hamberger, who exerted the most outstanding influence on the development and teaching of the specialty of otolaryngology

Acta Otolaryngol 83 40–46, 1977

NASAL OBSTRUCTION AND CARDIOPULMONARY SYSTEM IN CHILDREN

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Abstract 45 patients aged 6–18 years, with bilateral (17) and unilateral (28) obstruction of the nose, were analysed and divided into two groups in which nasal pressure ranged from 20 to above 60 mm H_2O The following examinations pertaining to pulmonary function were performed partial pressure of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}), pulmonary ventilation at rest and under charge (VC, FEV_1, MMF_{25-75}, IGV, FRC, RV, TLC, R_t) The investigation of the heart functions included the pulse rate, BP, and ECG at rest and under charge According to our findings, nasal obstruction in children has less influence on the lower respiratory tracts than contended in previous reports in the literature In no single case have we found any alterations in the function of the cardiovascular system at rest or during charge

Luscher (1930) reported from experimental and clinical observations that oral breathing disturbs the acid–alkali balance, i e there is a decrease in alkaline reserve in the blood due to pulmonary ventilation disturbance Šercer (1930, 1952) described the reflective influence of the nasal cav- on the lungs' homolateral side, as well as the tension of the bronchial musculature ·gura et al (1964, 1966, 1968, 1970, 1971, 1973) observed from their experimental and clinical data that nasal obstruction increases pulmonary (especially airway) resistance (R_A) and reduces compliance Their findings provided new insights into surgical interventions into the septum and nasal pyramid Cassisi et al (1971) recorded that patients who were treated with anterior and posterior packing for epistaxis, had hypoxemia with normocapnia, while hypoxemia with hypercapnia was observed in 20 patients with anterior and posterior packing as reported by Cook & Komorn (1973) However, 6 of these patients had a chronic obstructive pulmonary disease and 10, cardiovascular and hypertension complications In their most recent work, Cavo et al (1975) noted a significant depression in P_{O_2} and an elevation of P_{CO_2} following nasal packing in dogs These blood changes disappeared dramatically after packing was removed None of the above-mentioned changes in blood were recorded after packing in the 6 dogs which underwent total laryngectomy

The correlation between nasal obstruction and changes in cardiac function has only been described sporadically in literature Menashe et al (1965) reported hypoventilation and cor pulmonale resulting from obstruction of the upper respiratory tract Luke et al (1966) reported 4 cases of severe nasopharyngeal obstruction in which cardiorespiratory complications ranged from moderate cardiomegaly and hypertrophy of the right ventricle to right heart failure and pulmonary edema

Based on the material already published, we wanted to further examine the relationship between nasal obstruction and the cardiopulmonary system and conducted this investigation on children whose tissue structure is more elastic and in whom there is probably neither a pulmonary or cardiovascular lesion Three questions were analysed and tested (1) the effect of unilateral and bilateral nasal obstruction on the pulmonary system in children between 6 to 14 years, (2) the effect of nasal obstruction (unilateral and bilateral) on the values of respiratory gases in blood in children between 6 to 14

years, and (3) the effect of nasal obstruction on children's cardiac system during rest and activity

MATERIAL AND METHODS

For this investigation, we evaluated 45 patients between the ages of 6 and 18 who were operated on in our clinic for persistent breathing difficulties The majority of cases were 14 years and under They were divided according to local and rhinomanometric findings, depending upon the degree of obstruction, into two groups of 28 unilateral and 17 bilateral Each of these groups were then divided into two subgroups

Unilateral

(*a*) obstruction up to 30 mm H_2O (6 cases)
(*b*) obstruction over 30 mm H_2O (22 cases)

Bilateral

(*a*) incomplete (8 cases)
(*b*) complete (9 cases)

For each patient we analysed the pulmonary ventilation, blood gas values (P_{O_2} and P_{CO_2}) and the cardiovascular function

A double control of ventilation was always made with the expirograph (Godart) and the body plethysmograph (Godart) The functional residual capacity, i e the intrathoracic airway volume was determined by the body plethysmograph and by diluting the helium The patients were seated when pulmonary ventilation was measured The ergospirometric examination (15 cases) was carried out in the open system with Fleisch's pneumotachograph and the charge was given by mechanical ergocyclometry Children between the ages of 8 and 14 had a 50 W charge, while the remainder had a 100 W charge lasting 5 minutes on an ergometer bicycle with a rhythm of 50 revolutions per minute The electrocardiogram was measured before and during the charge

Blood gases drawn from peripheral blood capillaries were measured and analysed with the aid of an AME Radiometer (Copenhagen)

All examinations were made prior to surgery, the third and seventh day after surgery with packs in place and during the control period, 3–6 months later

For a control group, we analysed and compared the blood gas values of 20 healthy children between 6 and 14 years before and during their 24 hours anterior nasal packing

The second control group included 22 patients between 15 and 20 years who underwent surgery in our clinic but whose blood gas values were drawn and analysed in another clinic with the aid of another machine (M/Blood Gas Analyser 413) during and after removal of nasal packing

RESULTS

Pulmonary ventilation

In the complete bilateral nasal obstruction, the VC and FEV_1 values before surgery were significantly different from the values measured after surgery (Table I) We also recorded in the unilateral nasal obstruction that VC and FEV_1 values significantly varied before surgery and the seventh day after surgery with packs in place in comparison to the values measured during the control period 3 to 6 months later following removal of packs (Table II) The remaining parameters for measuring lung ventilation did not show any statistically important variations before and after surgery except for an elevated residual volume (RV) measured 7 days following surgery (with packs in place) which differed from the values obtained 3 to 6 months later after respiration had returned to normal (Table II)

The functional residual capacity (FRC) was increased in 4 patients with bilateral obstruction and in one with unilateral, but it still was within the limits of physiologic values, i e within two standard deviations according to Polgar

We recorded an elevated resistance (R_t) before surgery in only 2 cases with bilateral obstruction and 4 with unilateral obstruction over 60 mm H_2O, but this decreased by nearly 50% with removal of obstruction

Table I *Pulmonary ventilation in children and adolescents with bilateral nasal obstruction*

		N	Mean value	Statistical evaluation	T_0	d f	T (from Table)
VC	1 Before surgery	17	3 12±0 26	1 3	3 14	16	2 90 (0 01)
	2 7th day after surgery (with packs)	11	2 96±0 33	2 3	3 54	10	3 17 (0 01)
	3 3–6 months after surgery	17	3 26±0 25				
FEV_1	1 Before surgery	17	2 59±0 18	1 3	3 78	16	2 90 (0 01)
	2 7th day after surgery (with packs)	11	2.55±0 27	2 3	3 17	10	3 17 (0 01)
	3 3–6 months after surgery	17	2 79±0 20				
TT	1 Before surgery	17	83 94±1 87	1 3	1 39	16	2 12 (0 05)
	2 7th day after surgery (with packs)	11	84 33±2 56	2 3	1 14	10	2 23 (0 05)
	3 3–6 months after surgery	17	85 89±1 62				
MMF	1 Before surgery	17	3 36±0 26	1 3	2 088	16	2 12 (0 05)
	2. 7th day after surgery (with packs)	11	3 28±0 34	2 3	1 47	10	2 23 (0 05)
	3 3–6 months after surgery	17	3 51±0 27				
FRC	1 Before surgery	17	2 46±0 20	1 3	1 27	16	2 12 (0 05)
	2 7th day after surgery (with packs)	11	2 47±0 85	2 3	0 44	10	2 23 (0 05)
	3 3–6 months after surgery	17	2 32±0 20				
RV	1 Before surgery	17	1 08±0 09	1 3	0 58	16	2 12 (0 05)
	2 7th day after surgery (with packs)	11	1 17±0 12	2 3	0 16	10	2 23 (0 05)
	3 3–6 months after surgery	17	1 05±0 09				
R_t	1 Before surgery	11	3 61+0 36	1 3	1 28	10	2 23 (0 05)
	2 7th day after surgery (with packs)	6	3 83±0 73	2 3	1 03	5	2 57 (0 05)
	3 3–6 months after surgery	11	3 03±0 42				

Table II *Pulmonary ventilation in children and adolescents with unilateral nasal obstruction*

		N	Mean value	Statistical evaluation	T_0	d f	T (from Table)
C	1 Before surgery	28	3 24±0 22	1 3	2 31	27	2 06 (0 05)
	2 7th day after surgery (with packs)	28	3 18±0 22	2 3	3 23	27	2 78 (0 01)
	3 3–6 months after surgery	28	3 33±0 22				
EV_1	1 Before surgery	28	2 74±0 19	1 3	2 20	27	2 06 (0 05)
	2 7th day after surgery (with packs)	28	2 71±0 20	2 3	2 30	27	2 06 (0 05)
	3 3–6 months after surgery	28	2 82±0 18				
T	1 Before surgery	28	84 67±1 28	1 3	0 94	27	2 06 (0 05)
	2 7th day after surgery (with packs)	28	84 70±1 45	2 3	0 00	27	2 06 (0 05)
	3 3–6 months after surgery	28	84 71±1 25				
IMF	1 Before surgery	28	3 20±0 25	1 3	1 28	27	2 06 (0 05)
	2 7th day after surgery (with packs)	28	3 10±0 27	2 3	1 67	27	2 06 (0 05)
	3 3–6 months after surgery	28	3 31±0 24				
FRC	1 Before surgery	28	2 23±0 17	1 3	0 84	27	2 06 (0 05)
	2 7th day after surgery (with packs)	28	2 31±0 19	2 3	1 63	27	2 06 (0 05)
	3 3–6 months after surgery	28	2 19±0 16				
RV	1 Before surgery	28	1 03±0 09	1 3	0 85	27	2 06 (0 05)
	2 7th day after surgery (with packs)	28	1 08±0 09	2 3	2.24	27	2 06 (0 05)
	3 3–6 months after surgery	28	0 98±0 09				
R_t	1 Before surgery	15	3 69±0 21	1 3	1 93	14	2 14 (0 05)
	2 7th day after surgery (with packs)	15	3 80±0 34	2 3	1 43	14	2.14 (0 05)
	3 3–6 months after surgery	15	3 34±0 21				

Table III *Blood gas analysis in children and adolescents with unilateral nasal obstruction (P_{O_2} and P_{CO_2})*

			Statistical evaluation			
	N	Mean value		*T* (statistic)	d f	*T* (from Table)
(*a*) P_{O_2}						
Obstruction up to 30 mm H_2O						
1 Before surgery	6	87 00±1 29	1 2	1 61	5	2 57 (0 05)
2 3rd day after surgery (with packs)	6	84 17±0 95	1 3	2.00	5	2 57
3 7th day after surgery (with packs)	6	84 17+1 45	1 4	0 81	5	2 57
4 3-6 months after surgery	6	89 33±3 03	2 4	1.25	5	2.57
			3 4	1 20	5	2 57
Obstruction above 30 mm H_2O						
1 Before surgery	22	86 68±1 37	1 2	1 98	21	2 08 (0 05)
2 3rd day after surgery (with packs)	22	84 86±1 49	1 3	0 91	21	2 08
3 7th day after surgery (with packs)	22	86 04±1 14	1 4	2.03	21	2.08
4 3-6 months after surgery	22	89 27±1 27	2 4	2 44	21	2 08
	3		3 4	2 34	21	2 08
(*b*) P_{CO_2}						
Obstruction up to 30 mm H_2O						
1 Before surgery	6	44 82+1 53	1 2	0 55	5	2.57 (0 05)
2 3rd day after surgery (with packs)	6	45 77+1 42	1 3	0 24	5	2 57
3 7th day after surgery (with packs)	6	44 60±1 09	1 4	1 80	5	2.57
4 3-6 months after surgery	6	42 37±0 83	2 4	2.05	5	2.57
			3 4	1 96	5	2 57
Obstruction above 30 mm H_2O						
1 Before surgery	22	43 13+0 71	1 2	1 84	21	2 08 (0 05)
2 3rd day after surgery (with packs)	22	45 01+0 60	1 3	1 75	21	2 08 (0 05)
3 7th day after surgery (with packs)	22	44 35+0 70	1 4	1 72	21	2 08 (0 05)
4 3-6 months after surgery	22	42 16+0 45	2 4	5 24	21	2 82 (0 01)
			3 4	3 28	21	2 82 (0 01)

Analysis of blood gases

In patients with unilateral nasal obstruction up to 30 mm H_2O, no statistically important differences of P_{O_2} and P_{CO_2} values were observed either before or after surgery However in examinees with nasal obstruction above 30 mm H_2O, P_{O_2} and P_{CO_2} values measured the third and seventh day with packs in place were significantly different in comparison to the values drawn during the control period when normal nasal respiration had returned (Table III) There were no statistical differences in P_{O_2} and P_{CO_2} values in patients with incomplete bilateral nasal obstruction either before or after surgery, whereas there were differences in those with complete bilateral obstruction (Table IV)

Table IV *Blood gas analysis in children and adolescents with bilateral nasal obstruction*

	Number of patients	Mean value during obstruction	Mean value after obstruction	*T* (statistic)	Degrees of freedom	*T* (from Table)
Incomplete obstruction						
P_{O_2}	8	86 00±1 48	88 25±1 64	1 64	7	2 31 (0 05)
P_{CO_2}	8	44 66±0 99	42 32±0 77	1 94	7	2 31 (0 05)
Complete obstruction						
P_{O_2}	9	83 00±1 75	88 66±1 62	3 24	8	2.26 (0 05)
P_{CO_2}	9	44 75±1 54	41 00+1 13	2 27	8	2 26 (0 05)

Table V *Blood gas alteration in 20 healthy children following anterior nasal packing* (*Control Group I*)

	Number of patients	Mean value		T (statistic)	d f	T (from Table)
		Without packing	With packing			
P_{O_2}	20	90 61$\pm$1 16	87 27$\pm$1 39	2 26	19	2.10 (0 05)
P_{CO_2}	20	40 33$\pm$0 82	40 90$\pm$0 45	1 9	19	2 10 (0 05)

Table VI *Blood gas alteration in 22 adolescents during and after nasal obstruction* (*Control Group II*)

	Number of patients	Mean value		T (statistic)	d f	T (from Table)
		Without packing	With packing			
P_{O_2}	22	89 23$\pm$1 75	83 50$\pm$1 57	3 59	21	2 83 (0 01)
P_{CO_2}	22	37 27$\pm$0 60	37 81$\pm$0 60	0 95	21	2 09 (0 05)

In both control groups (20 and 22 examinees), we did record P_{O_2} changes during obstruction and after removal of packs, but the P_{CO_2} values did not vary significantly (Table V and VI)

Cardiovascular system

All patients had normal electrocardiograms before and during the 7 days' nasal packing Likewise no pathological changes in the electrocardiogram were recorded when the patients were exposed to the charge (ergospirometry and cycloergometry) The test was terminated for 2 patients due to subjective complications No ant changes in the blood pressure or rate were observed in the healthy group whose noses were packed for 24 hours (control group I) or in the operated group (control group II)

DISCUSSION

When analysing the effect of nasal obstruction on pulmonary ventilation in children, we concluded that there is a significant difference in VC and FEV_1 values during unilateral nasal obstruction ($P<0 05$) This was even greater in bilateral obstruction ($P<0 01$) However, it must be borne in mind that these are children in whom there may be a spontaneous increase in VC and FEV_1 values during growth and we should also not forget the possibility of a physiological variation between two consecutive measurements (4 5 and 8 8) The residual volume was significantly elevated only in cases of unilateral obstruction measured 7 days after surgery, i e when there was complete nasal obstruction We were particularly interested in the functional residual capacity and resistance whose elevation during nasal obstruction have been repeatedly reported in literature (Ogura et al , 1965, 1973, Unno et al , 1968, Ohnishi et al , 1971) We noted increased FRC and R_t in only 6 cases, while we did not find that changes in the other examinees were related to nasal obstruction In fact we even observed that FRC and R_t were sometimes lower during obstruction According to these results, nasal obstruction in children does not necessarily lead to essential functional changes in the lower respiratory tract, since children seem to have a good compensative mechanism and elastic tissue which can maintain a balance between nasal obstruction and pulmonary ventilation for a longer period of time

As regards to the second question about the relationship of arterial blood gases and nasal obstruction we observed that in unilateral nasal obstruction there were not significant value changes of P_{O_2} and P_{CO_2} in the blood In cases of complete bilateral obstruction, P_{O_2} and P_{CO_2} values showed significant changes before surgery as well as in the postoperative period following

nasal packing P_{O_2} changes were confirmed in the two control groups, while P_{CO_2} values varied during obstruction but not significantly When analysing the blood gases in relation to nasal obstruction, we can say that although these gases significantly varied during and after nasal obstruction, there were only 2 patients with complete bilateral obstruction and 2 with in complete who had before surgery P_{O_2} values below the baseline of 80 mm Likewise, in only some cases did P_{CO_2} values pass 45 mm

Thus blood gas changes are absolutely related to nasal obstruction caused by hypoventilation due to nasothoracic areflexy However, the compensative mechanism in children is able to pre vent hypoxemia and hypercapnia Drettner (1970) also reported that there are other investigators who did not find changes in the alka line reserve in children with nasal obstruction

In response to the third question, regarding the effect of nasal obstruction on the cardiovascular system, we did not observe in our 45 patients with nasal obstruction any significant changes during rest or activity We also con firmed in our control group of 20 non operated subjects whose noses were packed for 24 hours that during obstruction there were no significant changes in blood pressure or pulse rate Al though the possibility of a direct connection between the nose and heart through the trigeminal and vagus nerves is not excluded, our investigations have shown that nasal obstruction in children does not have a direct effect on the cardiovascular system That effect could be present indirectly when there is disturbed pulmonary circulation and repercussion on the right heart However, this could only occur when there has been complete nasal obstruction with severe breathing difficulties for a long period of time as described by some authors (Menashe et al, 1965, Luke et al, 1966) and a deficient cardio pulmonary system

In conclusion, we can surmise on the basis of our results that nasal obstruction does have an effect on the lower respiratory tract, but that it greatly depends upon the degree of obstruction, the reflexiveness of the organism, and the adaptability of the respiratory tract This has proved to be true in our own clinical experience, as nasal obstruction in one case may cause severe subjective sensations, while in another, symptoms may be absent for a long period of time

RESUMÉ

Nous avons analysé 45 patients ages entre 6 et 25 ans avec une obstruction nasale bilatérale (19) et unilaterale (26) divisés en 3 groupes avec une pression nasale entre 20 et au dessus de 60 mm H_2O Les fonctions pulmonaires et cardiales ont ete examinees en tranquillite et sous effort. Le groupe du contrôle est forme par des personnes saines (25) entre 18 et 20 ans Les résultats sont rapportes

ZUSAMMENFASSUNG

Wir haben 45 Patienten zwischen 6 und 18 Jahren mit beiderseitiger Nasenobstruktion bei 17 und einseitiger bei 28, die in 2 Gruppen geteilt waren, mit dem Nasen druck von 20 bis uber 60 mm H_2O, analysiert. Mit Bezug auf die pulmonäre Funktion wurden folgende Untersuchungen durchgefuhrt der partiale Sauerstoffdruck (P_{O_2}) und Kohlensauredruck (P_{CO_2}) im Blut, die Lungenventilation im Ruhen und bei Belastung (VK, FEV_1, MMF_{25-75}, IGV, FRC, RV, TLC, R_t) Von den Herzuntersuchungen wurden der Puls, RR und EKG im Ruhen und bei Belastung gemessen Unseren Befunden nach hat die Nasenobstruktion bei Kindern kleineren Einfluß auf die unteren Luftwege als bisherige Angaben in der Literatur behaupteten Wir fanden in keinem Fall im Ruhen oder unter Belastung Veranderungen in der Funktion des kardiovaskularen Systems

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(A) Reptilia Sauria
Lacerta muralis (newts) 10 animals

(B) Mammalia (i) Lagomorpha
Orytolagus cuniculus (rabbits) 10 animals
(ii) Rodentia
Mus musculus (mice) 6 animals
Cavia Porcelus (guinea pigs) 20 animals
(iii) Carnivora
Felix domesticus (cats) 4 animals
Canis familiaris (dogs) 6 animals

Total 56 animals

To study the morphology and histology in newts and mice, we beheaded the animals and embedded the heads in paraffin wax after decalcification In the bigger animals we proceeded in the same standard way to study the temporal bone in blocks intravital fixation with Wittmaack fluid and celloidin embedding In every case the blocks were sectioned in the frontal plane and the sections stained with hematoxylin and eosin

For the electronmicroscope study we proceeded in newts, mice and guinea pigs by beheading and sectioning the heads in two halves in the sagittal plane and immersion in fixative fluid Afterwards, with the aid of an operation microscope, we removed the organs in order to immerse them in the fixative fluid

In the bigger animals (rabbits, cats and dogs) the method was different. We proceeded by anesthetizing them with ketamin and then obtained the organs surgically with the animal still alive Subsequently we adopted the following procedure Under the surgical microscope we removed the cartilaginous envelope to immerse the organs immediately in glutaraldehyde with a common buffer system (phosphate solution according to Millonig pH 7 4) Post fixation was done with 1 % osmium tetroxide in the same buffer system, pH 7 2 Acetone was used for dehydration and the specimens were embedded by Espurr s method

Toluidine blue stained semithin sections (0 5–1 μm) were used as a control and selection of the areas to study

The ultrathin sections obtained with a LKB Ultratome with glass knives set at 45° were mounted on copper grids without a support film In order to obtain the contrast, we used double uranil–magnesium and lead citrate staining

The study was carried out by the use of a JEM-100 B electronmicroscope

Olfactory mucous membrane was sampled and studied in every case

RESULTS

Although our interest has been focused mainly on the ultrastructural aspects of the organ, we consider of interest to study the morphological and histological aspects as well, in order to select the different parts of the organ for the ultrastructural study

In morphological terms the organ was quite similar in the different species studied It is a small tubular structure lying within the nasal septum enclosed in a cartilaginous and bony capsule

In *Lacerta muralis* (newts) the organ is separated from the nasal cavities It opens into the mouth It is a roughly ovoid structure Its ventral part protrudes in its lumen, forming a mushroom shaped body like a turbinal process This body has a cartilaginous skeleton covered with a respiratory epithelium The vomeronasal epithelium lines its dorsal wall

In the mammals studied the sensory portion lines the inner wall but is sometimes formed like a horseshoe, lining the inner, upper and lower parts of the organ Only the outer walls are lined with a respiratory epithelium in these cases There are glands and vessels in the corium of this epithelium

In mice there is invariably a voluminous arteriola in the corium

In the mammals studied the duct opens into the nasal cavity, except in dogs where it opens into the nasopalatine duct

Histologically, both respiratory and sensory parts of the organ resemble the corresponding parts in the nasal cavity Both histologically and

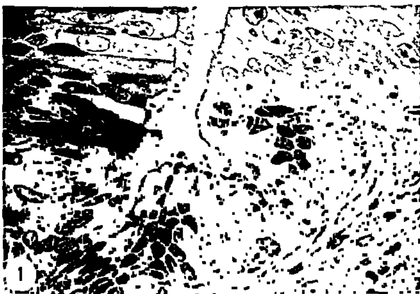


Fig 1 Lower commissure of the organ of Jacobson The borderline between the respiratory and the sensory epithelium is visible (*right*) Some ciliated respiratory cells can be seen (*left*) (Toluidine blue, semithin section)

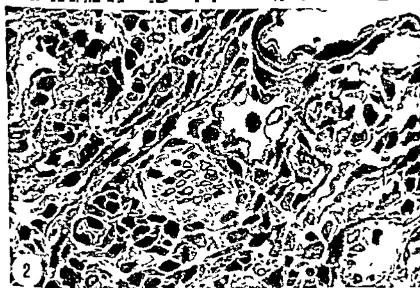


Fig 2 Detail of respiratory submucous chorion The cavernous body and nerves can be seen (Toluidine blue, semithin section)

ultrastructurally, we have been unable to detect any differences among the species studied We shall therefore describe extensively the organ of Jacobson only as found in the guinea pig We obtained more and better specimens from this animal

Histological study

In semithin sections we can observe the cavity of the organ lined by an epithelium that varies according to the walls examined In fact, we can distinguish two walls—inner and outer, and two commissures—upper and lower The outer (convex) wall is lined with a ciliated pseudostratified epithelium and the inner with vomeronasal epithelium Thus, this is the sensory part (Fig 1)

In both commissures there is an epithelium, flatter than the respiratory one, that slowly combines with the latter and abruptly ends at the beginning of the specialized epithelium The commissures (less often in the upper one) are traversed by gland ducts situated immediately below the mucosa In the stroma subjacent to the ciliated pseudostratified epithelium we find

a cavernous body where a multitude of vessels, nerves, and many smooth muscle fibres set in the interstices can be perceived (Fig 2)

Ultrastructure

1 *Pseudostratified epithelium* Here can be seen the peculiar features of this kind of epithelium in other locations, although without goblet cells Thus we can ascertain the presence of ciliated cells in whose apical surface there is an abundance of cilia, with their basal bodies and micro villi interspersed Among these cells, appear others having a very dense cytoplasm, without cilia on their surface, yet with microvilli (Fig 3) Ciliated cells present either rounded or elongated mitochondriae with a vesiculation process in some zones

2 *Commissure epithelium* This can be considered a transitional one, although it displays characteristics similar to the latter It is flatter and the quantity of ciliated cells decreases, while small secretory vesicles appear, having a low density to electrons in apical border of the cells (Fig 4) Close to the organ's epithelium proper, ciliated cells have disappeared but the pale and dark remain, with many smooth vesicles in their apical border, probably of a secretory nature

3 *Sensory epithelium* Its structural image depends on the site of sectioning as we saw in the semithin sections, it is not homogeneous throughout Wherever it grows thicker, it presents three types of cells some of them with tall, oval nuclei with thick chromatin accumuli (Fig 5), other, very large ones whose nuclei lie beneath those already mentioned, have a prominent central nucleolus (Fig 6) In the most basal area, a third layer of nuclei exists corresponding to triangular cells, and similar to those in the mucosa with a respiratory aspect (Fig 7)

This appearance changes on nearing the border zones where cells are less high, though they sometimes fill the entire thickness of the mucosa, these cells have some microvilli on their surface and moderate quantities of mitochondriae in their cytoplasm, below the nucleus, a glucogenic vacuole may occur

Subsequently, and as they reach the enlarged zone, the cells grow larger, and we then see that the cells with nuclei depressed terminate in characteristic apical poles with abundant microvilli, a bottle-neck with a profusion of centrioles and a large, subjacent bundle of mitochondriae that resemble the ellipsoid photoreceptors (Fig 8)

They are evidently sensory cells and we can now distinguish two types dark and light (Fig 9), although their apical poles are quite similar Among the dark cells, we have seen some with their apical pole ending in a club-like structure without microvilli, whilst others have the typical aspect as described earlier

In the enlarged zones, the cytoplasm adjacent to the nucleus has abundant rough endoplasmic reticulum and well developed Golgi apparatus (Fig 10) and as a peculiar feature there is a characteristic body originating from the smooth endoplasmic reticulum in a concentric manner not previously described (Fig 11) It is possible to find in these cells certain myelinic looking degenerative bodies (Fig 11) In the basal cytoplasm, a conspicuous smooth endoplasmic reticulum can also be discerned

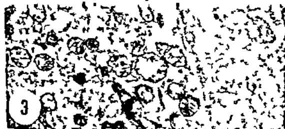
The cells with nuclei elevated present a simple and narrow apical pole without any enlargement or mitochondria or centriole structures We can detect in these poles only a few short, scattered microvilli They could be considered as supporting cells

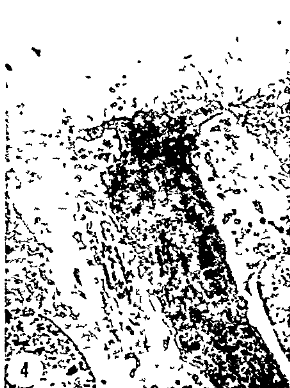
4 *Glands* The glands, located in the respiratory part of the organ, are characterized as forming small lumini where various cells with microvilli on their free surface congregate In the cytoplasm there are granules of varying electron density and abundant rough endoplasmic reticulum (Fig 12)

5 *Cavernous bodies* The associated cavernous bodies present abundant small, smooth muscle fibres of blood vessels (Fig 13) and myelinic or amyelinic nerves (Fig 14)

Comparative study between organ of Jacobson and olfactory mucosa

From this study we have been able to establish certain similarities and some profound differ-



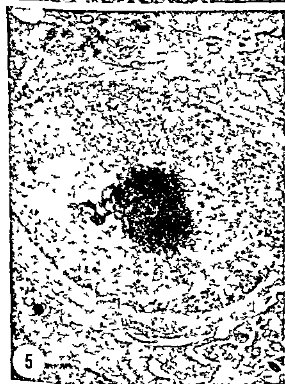




Fig 3 Dark and light ciliated cells of the respiratory part (Espurr ×15 000)

Fig 4 Secretory cells of the respiratory part Note that one of them is open permitting some vesicles to come out (Espurr ×8 000)

Fig 5 Rounded nucleus in the upper layer (Espurr 10 000)

Fig 6 Oval nucleus in the middle cell layer (Espurr 15 000)









Fig 7 Basal cells, with a capillary vessel (*bottom*) (Espurr, × 15 000)

Fig. 8. Sensory cells with microvilli, head, neck and abundant centrioles and mitochondria In the neck there are union complexes (Espurr, × 15 000)

Fig 9 Light cell between dark ones Both cell types have microvilli (Espurr, × 10 000)

Fig 10 Rough endoplasmic reticulum and Golgi apparatus in a sensory cell (Espurr, × 20 000)



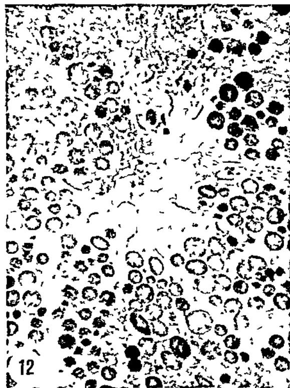






Fig 11 A very large, smooth endoplasmic reticulum under the nucleus in a sensory cell (Espurr, × 20 000)

Fig 12 Secretory cells forming the ductus lumen of a gland (Espurr, × 15 000)

Fig 13 Detail of a cavernous body with smooth muscle cells A capillary vessel is visible (*top*) (Espurr, × 8 000)

Fig 14 Myelinic and amyelinic nerve endings in the respiratory submucous chorion (Espurr, × 10 000)

structure homologous to the apical poles of the sensory cells of Jacobson's organ However, instead of hairs it has an important bundle of long microvilli In the olfactory vesicle, neurotubules 200 Å in diameter (Figs 15 and 16) and some mitochondria are constantly seen

The numbers of mitochondria and centrioles are more conspicuous in the organ of Jacobson than in the olfactory mucosa We cannot detect any secretory activity in the supporting cells of sensory mucosa of Jacobson's organ This activity has sometimes been detected in the olfactory supporting cells

Bowman's glands do not exist in the organ of Jacobson but we could detect (as we said earlier) a significant number of glands in the non-sensory part of the organ

DISCUSSION

We summarized our study of Jacobson's organ in the sketch shown in Fig 17 Our observations on the vomeronasal organ agree with the classic studies that have been carried out with light microscopy (Moulton & Beidler, 1967 among others) and with ultrastructural studies (Altner & Muller, 1968, Bannister, 1968, Kolnberger, 1971)

We were able to determine in the animals studied, that the sensory cells of the organ of Jacobson have a certain number of centrioles in the neck zone or dendrite zone, although these structures cannot be observed in others, such as *Anguis fragilis* (Bannister, 1968)

The accumulation of mitochondria is amazing They lie subjacent to the centriole location zone which resembles, as we said earlier, the common ellipsoid of photoreceptors, though in the vomeronasal organ these mitochondria are more sparse and their limits less precise

The presence of vesicles, arranged in the dendrites' most external part, was earlier described in a variety of animals (Bannister, 1965, Okano et al, 1967 Graziadei & Bannister, 1967, Thornhill, 1967) and they have been observed in our animals too, but in guinea pigs they have more regular limits than in others, they are light in density and their diameter is small The microvilli of their apical poles are prominent

A highly significant fact is the considerable hypertrophy of the smooth endoplasmic reticulum, which constitutes, in the guinea pig, a special body set in the basal cytoplasm, lateral to the nucleus zone We can hypothesize that these cells could form substances similar to the steroids (such as happens in suprarenal cortex cells and Leydic's luteal body of the testicle) or—on the contrary—they could play a part similar to the paraboloid photoreceptors

The secretory activity of the subtentacular cells is quite rare, but has been seen in all the animals studied Altner et al (1970) and Graziadei & Tucker (1968) have also described this feature

The glandular component of the vomeronasal organ resembles Bowman's glands, with typical dense bodies in the cell s apical pole, although it also has patent similarities to serous type glands in other locations

The contractile organ consists of both blood vessels and smooth muscle fibres, like those in other areas, often, mixed nerves with myelinic and amyelinic fibres and endings with pale and dense nucleic vesicles may be found among them These features are an indication of an organ with a contractile capacity, that has centrioles and mitochondrial structures whose functional interpretation and secretory capacity are difficult to determine

When we look at the design of Fig 17 we note a quite similar structure to the olfactory mucosa but the earlier described differences must be considered in the sense of a difference in functional properties

We must consider two main points of interest when comparing Jacobson's organ and the olfactory mucosa The first is the different structure of the apical pole and the second, the glands The main feature of the apical pole in the olfactory mucosa is the presence of the olfactory vesicle or "olfactory knob" (according to Vinnikov, 1974) By analogy we propose the

appellation "*vomeronasal vesicles*" for the apical pole of the sensory cell of Jacobson's organ

The olfactory vesicles form, with the microvilli and the mucous blanket, a "superstructure" which has a very important physiological role Vinnikov (1974) has described an olfactomotor phenomenon consisting in the protrusion of olfactory knobs into the lumen of the olfactory organ—or their retraction deep into the olfactory receptor layer—depending upon the functional state

Bronshtein (1964) has shown that antennae (hairs) in the olfactory cells of vertebrates are characterized by a continuous automatic motion, quite different in character and amplitude from the pulsation of ciliated cells

The addition of ATP to glycerinized cells with immobile hairs was accompanied by restoration of their motion The same happened when the olfactory cells were placed in an incubation medium containing nitroblue tetrazolium, due to blocking of the oxidative function of the mitochondria by formasan deposit on their cristae Subsequent addition of an ATP solution restored the automatic motion of the hair

These facts show (according to Vinnikov, 1974) that the factor responsible for the motion of antennae is the energy of the ATP generated by mitochondria of the olfactory knob

Returning to the vomeronasal organ, we may well be unable to consider this dynamic phenomenon as it does not have hairs, but microvilli Thus it is not possible to consider an active movement, but *rather a passive one*

We believe that this fact accounts for the functional differences between the two sensory organs However, we have seen some bare vesicles very close to the olfactory one, though without hairs We must mention, on the other hand, the existence of large bundles of mitochondria and centrioles These could be the basal bodies of the absent cilia, and the mitochondria the energy supply to these lost elements In line with this reasoning, it is possible to consider the organ of Jacobson a modified (not a degenerate) olfactory organ

The fact that the respiratory-like part of the organ exists, in close contact with the sensory one, gives this organ some independence and autonomy We must bear in mind the secretory and vasomotor activity of this part, which probably facilitates the contact between the stimulus with the receptor and the exchange of the material contained in the lumen of the organ

We consider the lack of Bowman's glands in the organ of Jacobson, as to be an unimportant difference between the two systems, as the amount of mucus it receives from the respiratory like part is insignificant In any case, Bowman's glands apparently develop at the expense of such displaced sectors of the respiratory epithelium (Vinnikov, 1974)

RÉSUME

L organe de Jacobson a été etudie dans les lezards, rats, cobayes, lapins et chiens par moyen de l inclusion en nitrocellulose, des sections sémi minces et microscopie électronique On a effectue l étude des divers élements de l organe dans les espèces etudiees et on a comparé la pars sensorielle de l organe mentioné avec l epithélium olfactif

ZUSAMMENFASSUNG

Das Jacobson-Organ wurde mittels Einschlusses in Nitrozellulose, mit halbfeinen Schnitten und Elektronenmikroskopie in Eidechsen, Ratten, Meerschweinchen, Kaninchen und Hunden untersucht Ein Studium der verschiedenen Elemente des Organs in den untersuchten Geschlechtern wurde durchgefuhrt und der Sinnesbereich des Organs wurde mit dem Geruchsepithel verglichen

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DISCUSSION

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PRIMARY NEURAL DISORDERS IN THE DEAF WHITE CAT COCHLEA

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Abstract Eleven white kittens were investigated from 2 days after birth up to an age of 4 months After their hearing ability or deafness had been tested electrophysiologically electron microscopic work was done at the level of the cochlea Previous histological data indicated that the hereditary process of degeneration begins at the epithelial and sensory elements of the cochlea, and that the neural degeneration is only a secondary and very slow process Results presented here indicate that, at least in some white cats this assumption needs to be modified Early failure in the myelination of the *lamina spiralis* fibres was noticed in most of the white kittens Furthermore, two kittens (7 and 16 days old) presented a complete degeneration of the spiral ganglion neurons, and signs of anterograde degeneration of their fibres going to the organ of Corti These primary neural defects indicate that hereditary hearing defects may directly affect both epithelio-sensory and neural structures

Most investigators of the deaf white cat agree that a primary degeneration of epithelial and sensory elements of the cochlea occurs during the first postnatal weeks, followed by a secondary and latter degeneration of the neural structures, lasting for months and even years (Howe, 1935, Bosher & Hallpike, 1965, 1967, Suga & Hattler, 1970, Mair, 1973, West & Harrison, 1973, Elverland et al, 1975) The same chronology is also said to be found in other cases of hereditary deafness, e g the mouse (Gruneberg et al, 1940, Mikaelian et al, 1974), the Dalmatian dog (Johnsson et al, 1973, Mair, 1976), and man (Bergsma & Brown, 1971) In agreement with these findings, we previously described (Rebillard et al, 1976) well preserved neural structures within a degenerated white cat cochlea We pointed out the great number of remaining nerve fibres among unrecognizable epithelial elements of the organ of Corti, and held those fibres to be responsible for the residual hearing ability in agreement with Suga & Hattler's (1970) hypothesis of direct fibre stimulation

This work has been supported by grants from INSERM and DGRST

Nevertheless, there have been a few reports on a degenerative process affecting primarily the spiral ganglion neurons and fibres Alexander & Tandler (1905) first reported such a case in a white kitten Later, Saxen (1934) and Altmann (1950) in man, and recently Igarashi et al (1972) in the Dalmatian dog, called attention to a possible hereditary defect affecting neurons first or, at least, at the same time as the other cochlear structures On the basis of these data and on a previous observation of a 4-month old white cat cochlea, characterized by a surprising number of unmyelinated fibres (Rebillard et al, 1976) we decided that a more extensive study to verify the occurrence of such primary neural disorders was justifiable, our first results are presented here

MATERIAL AND METHODS

Eleven white kittens from 2 days after birth to 4 months of age were used After determining behaviourally any possible deafness, electrophysiological responses were tentatively recorded under light Nembutal anaesthesia at various levels of the auditory pathway (i e gross cochlear potentials, inferior colliculus and auditory cortex evoked responses) Histological techniques adapted from Spoendlin were applied Essentially, they consisted of an in vivo fixation of the cochlea by glutaraldehyde-osmic acid

perfusion, and Spurr embedding Thin sections, mounted on Parlodion-coated grids and counterstained with uranyl acetate and lead citrate, were checked with a Philips 300 or a Jeol 100C electron microscope

RESULTS

Early signs of myelination defects in white kitten cochlea

The fibres within the basal *lamina spiralis* of a normal kitten cochlea begin to be myelinated as early as the week before birth (Pujol & Hilding, 1973) Four days before birth the basal portion of the cochlea presents an advanced stage of myelination affecting the majority of nerve fibres (Figs 1, 2) This process is completed during the second postnatal week when more than 20 myelin lamellae surround the fibres (Fig 3)

No white kittens were investigated before birth, consequently, we missed the very first stages of myelination But as early as the first week after birth, hearing white kittens (2, 4 and 7 days old) presented cochleae with signs of abnormal myelination The proportion of unmyelinated fibres was larger than in normal coloured kittens (Figs 4, 5), however, the num- [illegible] of myelin lamellae surrounding other fibres [illegible] quite normal for this age (see also Fig 3), and other structures of the cochlea were not damaged This myelination defect was not related to the axonal diameter, as it affected fibres of all sizes

An abnormal proportion of unmyelinated fibres was also encountered in older white cats (16 and 26 days, 3 and 4 months old) with some residual hearing ability (Figs 6, 7) In deaf kittens these unmyelinated fibres were found together with degenerating ones—that degeneration appearing, at first, like untighted and irregular myelin lamellae (Fig 8)

Early degeneration of spiral ganglion neurons in some white kittens

In 2 cases (7- and 16-day-old kittens), we noticed an already complete deafness associated with major abnormalities of the spiral ganglion Neurones appeared almost completely degenerated, with a clear and empty cytoplasm, a non myelinated membrane, and a small nucleus with dispersed chromatin (Figs 9, 10), features never previously described before several months after cochlear degeneration (Fig 11)

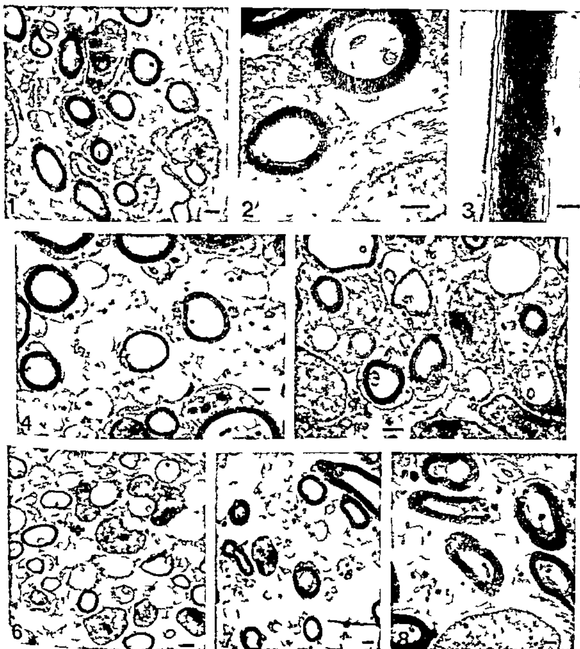
Another finding in these animals was the appearance of preganglionar fibres Close to the cell body, they were completely degenerated (Fig 12), only a "contour" and some myelin residuum was found As we approached the *habenula perforata* these fibres presented less pronounced signs of degeneration and had a well preserved myelin sheath and some axoplasmic inclusions (Fig 13) Here there were characters of an anterograde process of degeneration affecting first the soma of the neurons and reaching the fibres secondarily It is of interest to note that in these kittens, the sensory cells of the organ of Corti appeared in a much better state of preservation than nerve elements (Fig 14)

DISCUSSION

From a previous observation (Rebillard et al, 1976) and those reported here, it now seems clear that neural elements of the white cat cochlea may be affected from the very onset of the hereditary syndrome

Unmyelinated fibres

In most white kittens, even when deafness is not yet complete, a large number of unmyelinated fibres are found A quantitative study, now under way, will specify the exact proportion of such fibres as well as any possible reduction in the total amount—myelinated and non myelinated—of *lamina spiralis* fibres As optic microscopy cannot reveal the presence of unmyelinated fibres among well myelinated ones, no such observation has previously been made by researchers on hereditary deafness in white cats, using this method alone (Bosher & Hallpike, 1965, 1967, Mair, 1973)



Figs 1 2 Lamina spiralis fibres from the basal coil of the cochlea of a normal coloured kitten 4 days before birth Myelination already affects the majority of the nerve fibres Calibration for all the figures = 1 μm, except Fig. 3 = 0 2 μm.

Fig 3 Normal myelin sheath (more than 20 lamellae) of a *lamina spiralis* fibre from a 7-day-old white kitten cochlea

Figs 4 5 Numerous unmyelinated fibres found inside the *lamina spiralis* of hearing white kittens aged 4 days (Fig. 4) and 7 days (Fig. 5)

Figs 6 7 Unmyelinated fibres from the hearing cochlea of a 16-day-old (Fig. 6) and a 4-month-old (Fig. 7) white kitten with monaural deafness

Fig 8 Unmyelinated fibre and degenerating ones in the *lamina spiralis* of a 1-month-old deaf white cat.

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to formulate the hypothesis that such abnormality is a part of the general syndrome related to neural crest hereditary impairment (Bergsma & Brown, 1971) This primary defect may be of minor consequence for hearing, in comparison with the drastic degenerative process later affecting the sensory cells or the spiral neurons

Early neuronal degeneration in the spiral ganglion

At the level of the spiral ganglion, previous data indicated a probable secondary degeneration of the neural elements of the white cat cochlea (Howe, 1935, Bosher & Hallpike, 1965, West & Harrison 1973) Mair, in the white cat (1973) and the Dalmatian dog (1976), made a statistical study based on neuron counts and densities along the entire cochlea at different ages He reported that a very slow process of degeneration occurred, beginning after the completion of the degeneration of the organ of Corti and lasting several years The majority of our observations agreed well with this general statement (Rebillard et al, 1976) but the process seems much more variable and often more rapid than that described by Mair Perhaps one should take into account the fact that he worked on selected pure strains in which individuals presented a remarkable continuity

The 2 cases which showed a complete degeneration of the spiral ganglion during the first weeks of post natal life, must be interpreted in an entirely different way, for here the neural degeneration was clearly anterograde and other cochlear structures were in a better state of preservation Actually, hearing loss seemed to depend first on nervous abnormalities

These findings tend to consider the degeneration of the white cat cochlea as a process affecting both sensory and neural structures with a variety of features and a very variable timing In order to correlate hereditary deafness occurring in animals, such as white cats, with some of the congenital hearing losses in man more information is needed, such as an analysis of the various ways in which the cochlea stops functioning and the determination of a possible environmental factor (Bosher & Hallpike, 1967) as the link in these diverse impairments

RESUME

L expérimentation a porté sur 11 chats blancs, agés de 2 jours à 4 mois Après controle electrophysiologique de leurs capacités auditives ou de leur surdité les cochlees ont eté etudiées en microscopie electronique Des travaux anterieurs indiquaient que le processus de degénérescence hereditaire commençait par les elements épithéliaux et sensoriels pour atteindre secondairement et tres tardivement les structures nerveuses Les résultats presentes ici attenuent cette affirmation En effet des defauts de myelinisation des fibres laminaires sont releves chez les chatons blancs alors meme que leur audition n est pas encore affectée De plus, sur 2 animaux de 7 et 16 jours une degenerescence complete du ganglion spiral a éte rencontree tandis que les fibres préganglionnaires presentaient une degenerescence nettement antérograde Ces observations montrent que les deficits héréditaires peuvent atteindre directement les elements epithelio-sensoriels comme les élements nerveux de la cochlee

ZUSAMMENFASSUNG

Elf weiße Katzen wurden in einer Zeitspanne von 2 Tagen bis 4 Monaten nach der Geburt untersucht Nachdem das Horvermogen beziehungsweise die Schwerhorigkeit elektrophysiologisch getestet war, wurden die Schnecken elektronenmikroskopisch untersucht Fruhere histologische Befunde zeigten, daß der hereditäre Degenerationsprozeß an den Sinnesepithelien der Cochlea beginnt und die neurale Degeneration erst sekundar und langsam auftritt Durch die in dieser Arbeit gezeigten Befunde muß diese Ansicht allerdings etwas modifiziert werden Eine fehlende Bemarkung der Nervenfasern der Lamina spiralis ossea wurde bei den meisten weißen Katzen gefunden Zwei Katzen (7 und 16 Tage alt) zeigten eine komplette Degeneration der Spiralganglien und Zeichen einer anterograden Degeneration der Nervenfasern zum Cortischen Organ Diese frühen neuralen Defekte weisen darauf hin daß der hereditäre Degenerationsprozeß primar und direkt sowohl die Sinnesepithelien als auch die Neuronen erfaßt.

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DISCUSSION

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A SURVEY OF THE CYTO-ARCHITECTURE OF THE ORGAN OF CORTI

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INTRODUCTION

Modern methods of structural research such as transmission electron microscopy (TEM), scanning electron microscopy (SEM) and freeze-fracturing, as well as techniques using tracer substances like thorothrast, ferritin, horseradish peroxidase or radio nucleids have been used by our group in studies on the inner ear Some of the results will be published by Bredberg and by Angelborg in this volume

The present paper deals mainly with the general morphology of the organ of Corti It is based on an extensive study of the inner ear Several richly illustrated papers are published or are under publication dealing with similar subjects (cf Engstrom et al, 1966, Engstrom & Ades, 1973, Angelborg & Engstrom, 1972, Inner ear studies I, II Acta Otolaryngol, Suppl 301, 1972, Suppl 319, 1974) All these publications contain descriptions of the normal and pathological morphology of the inner ear

The sensory cells of the organ of Corti are of two types, inner and outer hair cells surrounded by supporting cells SEM permits a very informative, almost three-dimensional visualization of both the surface of the organ of Corti and of its interior As earlier pointed out, it is possible by microdissection to open the interior of Corti s organ to expose the basilar membrane, the supporting cells, nerve fibres and nerve endings In this way the interrelation of different sensory cells and supporting elements can be readily observed and nerve fibres and nerve endings studied over rather long distances A thorough knowledge of the normal morphology is of the utmost importance for the evaluation of modifications caused by ototoxic agents, noise, or aging These are problems which we are constantly concerned with

Present preparation techniques and resolution in the microscope visualizes modifications of individual hairs or groups of hairs (Fig 1) It is also possible to observe healing processes after damage and cells present or lost Here the combination of SEM and TEM is usually necessary to evaluate the extent of damage

Microdissection of the organ of Corti has been used by us for a long time It is possible to make a cross section or cross fracture of the organ of Corti and it can then be seen how nerve fibres, nerve endings and supporting cells are interrelated (Figs 2, 4) It is at such studies interesting to see how well early scientists such as Retzius (1884) or Held (1926) interpreted their findings and depicted them

In the normal organ of Corti of mammals the sensory cells are, as seen from the surface, arranged in a beautifully regular pattern carefully described by Retzius (1884), by Neubert (1952–1960) and by Engström et al (1966) This arrangement can be seen in Figs 1 and 2 Even when only phase-contrast microscopy was used it could be seen that the sensory hairs were arranged like a W and that this W had a different form in different cochlear coils TEM and SEM have clearly shown that the angle between the rows of hairs increases from upper portions of the cochlea towards the base Thus there seems to be a relation between frequency response region and the arrangement of the hairs on the outer hair cells The hairs on inner hair cells



ıg *1* In these three figures the pattern of sensory cells n be seen in the guinea p g In A the pattern is partly od fied (*arrow*) due to exposure to noise In C the angle in the *H* is more than 100 indicating that the cells are from the lower half of the cochlea (A) × 300 (B) × 1 400 (C) × 10 000



Fig. 2 (A) The organ of Corti from a guinea pig with one row of inner hair cells (*I*) and three rows of outer hair cells (1, 2, 3). In the tunnel of Corti (*TC*) some nerve fibres can be seen. ×750. (B) The organ of Corti has been opened to visualize the interior of the tunnel of Corti. 1, 2 and 3 are the three rows of outer hair cells. *STB* is the spiral tunnel bundle. The black arrow indicates afferent *nerve* fibres at the bottom of the tunnel while white arrow indicates *efferent* fibres (mainly). ×900

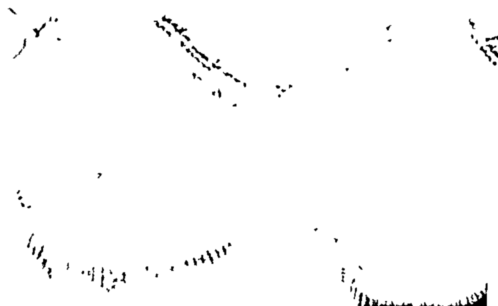


Fig 1 In these three figures the pattern of sensory cells can be seen in the guinea pig In A the pattern is partly modified (*arrow*) due to exposure to noise In C the angle in the W is more than 100° indicating that the cells a from the lower half of the cochlea (A) ×300, (B) ×1 40 (C) ×10 000



Fig 4 (A) Microdissection shows the interior of the organ of Corti with nerve endings (*Ne*) at outer hair cells (*OHC*) nerve fibers (*arrow*) and how Deiters cells enclose the nerve fibers (A) ×5 500 In B the branching of an efferent nerve fiber can be seen The relation to the Deiters cells is also seen (B) ×6 500

cochlear capsule was thinned down with a fine, round, diamond burr and the bone removed The organ of Corti associated with the osseous spiral lamina was removed in segments according to the surface specimen technique (Engström et al, 1966, Bredberg, 1968) The specimens were further dehydrated in acetone, from 70% to 100% and critical point dried in carbon dioxide in a Polaron E3000 critical point drying unit In the dry state the specimens were dissected using specially sharpened watchmakers forceps The fluid spaces could be opened and the organ separated in various planes, thus exposing the nerve structures inside the organ of Corti The specimens were mounted on a stub with double-sided adhesive tape and studied in a Jeol JSM-U3 scanning electron microscope Before specimens were studied in the microscope they were coated with a layer of gold in a Polaron SEM coating unit E5000 by the principle of sputtering

RESULTS

By a careful dissection of the organ of Corti it is possible to visualize the various fluid spaces, such as in cross section (Fig 1) or in other planes (Figs 2–8) This makes it possible to tudy the nerve fibres exposed in the spaces

The efferent fibres cross the tunnel of Corti and the space of Nuel, running freely above the floor of the tunnel (Figs 3, 5) They emerge between the inner pillars in the medial (inner) angle of the tunnel Some fibres turn in a spiral (longitudinal) direction and form the spiral tunnel bundle Other fibres cross through this bundle and form groups of fibres crossing the tunnel space (Fig 3) together with fibres which emerge from the spiral tunnel bundle In the space of Nuel many of the crossing efferent fibres divide once or twice before they disappear in between the Deiters cells (Fig 4) A few of the fibres directly contact nerve endings on the hair cells, whereas most of them disappear under the Deiters cells before they end in nerve endings on the sensory cells (Figs 5, 6, 7)

The afferent nerves emerge between the inner

Fig 1 Cross section through the organ of Corti The surface is formed by the reticular lamina in which the hair bearing end of the outer hair cells is suspended On the surface, three rows of bundles of the outer hair cells and one row of the inner hair cells are seen The cell bodies of the outer hair cells are supported by the Deiters cells from below The space of Nuel is to the right of the outer hair cells and the outer tunnel is to the left The fibres of the basilar membrane are bent in a wave form and on its underside are cells of the tympanic cover layer The tunnel of Corti is far to the right compressed by artifact, and containing the spiral tunnel bundle Guinea pig ×1 500

Fig 2 The tunnel of Corti and the space of Nuel seen from below The basilar membrane and crossing nerve [illegible] basilar membrane and the pillar cell bodies are seen in the centre of the figure Above are the inner pillars and spiral tunnel bundle Guinea pig ×2 000

Fig 3 The tunnel and the space of Nuel seen from below after removal of the basilar membrane Efferent fibres in the spiral tunnel bundle are seen in the upper part of the figure Crossing efferents between the outer pillars reach the upper portion of the Deiters cells in the space of Nuel below Parallel fibres below are afferent fibres in the first outer spiral bundle Rabbit lower basal coil (120° 180°) ×2 500

Fig 4 Higher magnification of the same area of the cochlea as in Fig 3 Many of the efferent fibres are dividing once or twice just after passing between the outer pillars in the space of Nuel Rabbit, lower basal coil (120 –160) ×7 000

Fig 5 The space of Nuel has been opened and its medial wall the outer pillars folded upwards (upper half of the figure) exposing the lateral wall (lower half of the figure) with the first row of outer hair cells and the Deiters cells. Nerve fibres in the space between the pillars are crossing efferent fibres which were divided at the dissection (white arrows upper half of the figure) The other ends of these fibres are seen in the area below the outer hair cells (white arrows lower half of the figure) The afferent fibres have crossed the tunnel on its floor and run between the outer pillar cell bases to the space of Nuel where they slowly descend on the pillars (black arrows, upper half of the figure) in a direction towards the basal end of the cochlea until they reach the bottom of the space and slowly climb on the lateral wall on the Deiters cells forming the outer spiral bundle (black arrows, lower half of the figure) Note nerve endings on the lower and middle portion of the hair cells Rabbit, upper basal coil (210 360) 1 000

Fig 6 Lateral wall of the space of Nuel with the first row of outer hair cells and the Deiters cells The afferent nerve fibres in the first outer spiral bundle run for a considerable distance (at least 0 5 mm) slowly climbing the Deiters cells Torn nerve fibres in the upper part of the figure are crossing efferent fibres (*arrows*) Note nerve endings on the hair cells. Rabbit upper basal coil (210 360) 2 900



Fig 1

Fig 2

Fig 3



Fig 4

Fig 5

Fig 6

Fig 7



Fig 8

pillars under the efferent spiral tunnel bundle and run on the floor of the tunnel when crossing this space Sometimes the fibres run depressed in the pillar cells on the floor, but traces of the fibres can always be seen as a row of small microvilli along the furrow formed by the nerve fibre The fibres, however, only run short distances buried in the pillar cell bases before they disappear between the bodies of the outer pillar cells In the guinea pig the fibres cross straight over the space of Nuel to its lateral wall and enter the outer spiral bundle where they run spirally (longitudinally) for at least 0 5–0 7 mm In the rabbit, however, when reaching the space of Nuel the afferent fibres run spirally (longitudinally) on the outer pillar bases, slowly descending in parallel course until they reach the bottom of the space (Fig 5) Here the fibres at times form a small bundle before they slowly climb the lateral wall of the space of Nuel Thus the fibres forming the outer spiral bundle consists of two fascicles, one on the pillar cells and one on the Deiters cells (Fig 5) The fibres may run on 5–15 pillar cells in parallel course On the Deiters cells the fibres run very regularly parallel and only occasionally cross each other In the rabbit it is possible in the SEM to evaluate these fibres throughout the different coils, whereas in the guinea pig the pattern seems to be more complex and more hidden by covering Deiters cells In the rabbit the fibres lie for long distances only touching the Deiters cells and seem to be held in place by small, staple like processes from the Deiters cells When the fibres have reached the upper portion of the Deiters cells they disappear under a complex pattern of cells and fibres formed by the Deiters cells and the efferent fibres (compare Figs 6 and 8, in Fig 8 the efferent fibres have degenerated)

Fig 7 Nerve endings on the basis of three outer hair cells A group of nerve endings contact two sensory cells Deiters cells partly cover the lower poles of the sensory cells and some nerve endings Guinea pig upper third coil × 10 000

Fig 8 Lateral wall of the space of Nuel 10 days after the efferent nerves to the cochlea had been cut by dividing the vestibular nerve All nerve endings above the Deiters cells on the outer hair cells have degenerated indicating their efferent nature (compare Fig 6) The afferent fibres in the outer spiral bundle (lower part of the figure) are intact Rabbit upper basal coil (290 370) 2 300

On the sensory cells large efferent nerve endings are seen at the base of the cells and in between the Deiters cells Often the endings are arranged in groups which sometimes contact two sensory cells In the rabbit a special type of nerve ending is frequently found on the sensory cell side and at times high up on the cell (Figs 5, 6) This type of nerve ending is small, mostly like a small rounded bud, though some fibres end with a very small ending without a swelling In the guinea pig these endings are less common, about one on every fifth to tenth first row outer hair cell In the chinchilla they seem to be even more sparse These nerve endings disappear after cutting the efferent nerve supply to the ear, thus indicating their efferent nature (Fig 8) These endings will be discussed more in detail in a forthcoming publication

DISCUSSION

The purpose of this report has mainly been to present the SEM method of studying the fluid spaces within the organ of Corti It has been demonstrated that it is possible to evaluate large portions of the organ of Corti and to study in detail the course of the nerve fibres It has not been possible, so far, to obtain a direct visual differentiation in the SEM between afferent and efferent innervation Instead this differentiation has been made by cutting the efferent fibres where they run in the vestibular nerve as described by Iurato (1962), Spoendlin & Gacek (1963) and Smith & Rasmussen (1963) It has not so far been possible to study the innervation of the inner hair cells as there is no fluid space in this region of the organ of Corti exposing the nerves or the cell body of the inner hair cell

The tunnel nerves and the nerves in the space of Nuel are easily accessible for study It has also been possible in some specimens to expose the second and third outer spiral bundles The region directly under the sensory cells and the

connections between the spiral bundles are more difficult to get access to with this method These areas will be studied with transmission electron microscopy Further studies will also include the study of inner ears with small, localized lesions in order to evaluate the length of spiral distribution of nerve fibres

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Die Innervation innerhalb des cortischen Organes wurde an Meerschweinchen, Chinchillas und Kaninchen unter dem Rasterelektronenmikroskop studiert Die Präparate wurden fixiert, dissikiert, getrocknet mit der Kritischen Punkt Methode und mit Gold belegt Die schliessliche Mikrodissektion der Präparate geschah in trockenem Zustand in den Ebenen der Flussigkeitsraume Der Verlauf der efferenten und afferenten Nervenfasern wird beschrieben Die efferenten Fasern erreichen die äusseren Haarzellen, indem sie den Tunnelraum uberqueren und zwischen den äusseren Pfeilern verlaufen und sich mehrmals in Nuels Raum teilen Die afferenten Fasern überqueren den Boden des Tunnels und wenden sich basalwarts in den ausseren Spiralbundeln, in denen sie zumindest 0,5–0,7 mm verlaufen, bevor sie sich mit den äusseren Haarzellen verbinden Nach Unterbrechung efferenten Nerven verschwinden die kleinen Nerdigungen an die Seite jeder funften bis zehnten äusseren Haarzelle, was fur den efferenten Charakter der Nervenendigungen spricht

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DISCUSSION

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FREEZE FRACTURE STUDY OF THE CELL JUNCTIONS IN THE UTRICLE AND SACCULE

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Abstract The maculae sacculi and utriculi of the chinchilla vestibular labyrinth have been studied by freeze-fracture method In the replicas extensive *zonulae occludentes* have been found between sensory and supporting cells at the endolymphatic surface *Gap junctions* are located between the supporting cells Some intramembranous specializations of the *synaptic regions* are described in both types of the sensory cells

The fine structure of the vestibular labyrinth has already been extensively studied by thin section electron microscopy (reviews by Smith, 1967, Wersäll, 1967, Spoendlin, 1970, Engström et al, 1972, Wersäll & Bagger-Sjöbäck, 1974) In the present paper some features revealed in the utricle and saccule by the freeze fracture technique will be described The results have been compared with those obtained in the cochlear duct by using the same method (Reissner membrane, Franke et al, 1975, vascular stria, Reale et al, 1975, organ of Corti, Iurato et al, 1976*a*, *b* Gulley & Reese, 1976*a*, *b*)

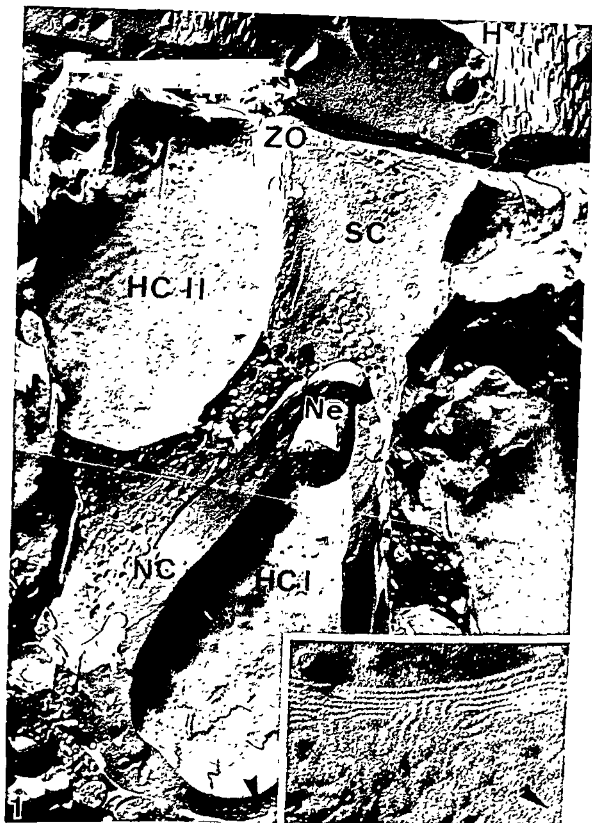
MATERIAL AND METHOD

Twelve adult chinchillas were anesthetized with an intraperitoneal injection of sodium pentobarbital (35 mg/kg) The inner ear was briefly prefixed by perilymphatic perfusion, isolated and left 4 to 10 hours at 0–4 C in the same fixative used for perfusion The fixative solution contained 2% formaldehyde, 1% glutaraldehyde, 0 1 M cacodylate buffer (pH 7 3–7 4) and 25 mg% $CaCl_2$ The ampullae of the semicircular canals, the saccule and utricle were dissected and transferred for 90 min in a 30% solution of glycerol in saline The specimens were oriented on holders, rapidly frozen in Freon 22 (monochlorodifluoromethane) at about −150 C, transferred in a Balzers BA360M apparatus (Balzers AG, Liechtenstein), fractured at 110°C and shadowed with platinum-carbon The replicas were cleaned with hypochlorite bleach and chromic acid, washed repeatedly in distilled water, collected on Formvar-carbon membranes (Dowell, 1964) and examined in Siemens Elmiskop Ia and 101 electron microscopes

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RESULTS

In the apical region of the sensory and supporting cells of the maculae sacculi and utriculi, where in thin sections the so-called reticular membrane has been described (Smith, 1967), extensive *zonulae occludentes* are evident (Fig 1) Their strands appear as ridges on the cytoplasmic face and as grooves on the external face of the split plasma membrane In the more apical part of the junction the strands run close to each other, parallel with the endolymphatic surface and with only few interconnections (Fig 2) Deeper, the interconnections are more numerous, resulting in a network with tight meshes between sensory and supporting cells (Fig 2) Between adjacent supporting cells the meshes extend still deeper but they make only a loose,


H
ZO
SC
HC II
Ne
NC
HC I
1

incomplete network Also maculae occludentes and isolated strands were observed here Within the lower meshes of the zonulae occludentes and below these, *desmosomes* are present They have been described in this location by Smith (1967) and by Engstrom et al (1972) and appear as large clusters of different size particles covering both faces of the split plasma membrane

Gap junctions were observed on fracture faces of the supporting cells They are characterized by assemblies of particles on the cytoplasmic face of a supporting cell plasma membrane and of pits on the complementary external face of the plasma membrane of the adjacent supporting cell Generally they are small apically, larger near the basal membrane

In both types of sensory cells afferent and efferent *synapses* were identified in the replicas In the perinuclear region the external face of the plasma membrane of the hair cells type I is almost completely covered by 11 nm roundish particles (Fig 3) Only small areas are devoid of particles These areas correspond to clusters of particles which are present on the cytoplasmic face of the same membrane at the level of a synaptic bar Opposed to these particle clusters, aggregates of particles were also observed on the external face of the adjacent nerve chalice plasma membrane The cytoplasmic face of the chalice plasma membrane is covered by numerous scattered particles 8–13 nm in diameter

Fig 1 A hair cell type I (*HCI*) surrounded by its nerve chalice (*NC*) has been fractured at the level of its neck (*Ne*) hair cells type II (*HCII*) supporting cells (*SC*) and in the lower left corner, nerve fibres in a macula utriculi are visible *E* = endolymphatic space *H* hairs Cleaving within the plasma membrane of some cells exposes the structure of the zonulae occludentes (*ZO*) fracturing across the cells illustrates the internal structure of the macula components as in a thin section ×9 000.

The endolymphatic surface of the macula is located at the top of each picture The direction of the carbon-platinum shadowing is indicated by the arrowhead at the right lower corner

Fig 2 Part of the zonula occludens between a hair cell type I and an adjacent supporting cell This and the external half of the sensory cell plasma membrane have been cleaved out by fracturing On the cytoplasmic face of the hair cell membrane the strands of the zonula occludens are visible ×48 000

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The cytoplasmic face of the plasma membrane of the hair cells type II is characterized by particles of approximately 11–12 nm in diameter These particles are arranged in single and seldom double rows of varying length, orientation and distance from each other (Fig 4)

The efferent terminals to the hair cells type II are identified, when cross fractured, owing to their numerous synaptic vesicles In *en face* views of the presynaptic membrane they appear as indentations (cytoplasmic face) or as protuberances (external face) corresponding to synaptopores or vesicle attachment sites (Pfenninger et al, 1972) Large particles are usually associated with the cytoplasmic face of the presynaptic membrane The cytoplasmic face of the postsynaptic membrane presents clusters of particles which are apparently facing the efferent terminals

DISCUSSION

The structure and extension of the zonulae occludentes found in the vestibular sensory areas are similar to those described by Iurato et al (1976*a*) and by Gulley & Reese (1976*a*) in the reticular membrane of the organ of Corti, using the same technique and the same animal However, they show a very complex structure between sensory and supporting cells, they are still deeper but apparently looser between the supporting cells

The synaptic area of the hair cells of type I is confined to the perikaryal region (Hamilton, 1968) In the replicas this region is characterized by the very extended covering of particles evidenced on the external face of the hair cell type I plasma membrane In this region are also present the synaptic bars and their surrounding vesicles The intramembrane specializations underlying the synaptic bars—assemblies of particles on the cytoplasmic face of the presynaptic membrane and on the external face of the postsynaptic membrane—are similar to those described by Gulley & Reese (1976*b*) in the organ of Corti between inner hair cells and afferent nerve endings


A
C
B
SC

En face views of the efferent terminals on the hair cells of type II show almost the same specializations of their synaptic plasma membrane as elsewhere in cholinergic synapses (Heuser et al, 1974, Peper et al, 1974, Rash & Ellisman, 1974, Ellisman et al, 1976) and in the organ of Corti (Gulley & Reese, 1976*b*) There are synaptopores and intermingled large particles on the cytoplasmic face of the plasma membrane In the opposed post-synaptic membrane, particles are visible on the cytoplasmic face Neither synaptopores nor specializations of the post-synaptic membrane, however, show any pattern which instead characterizes the active sites of the motor end plates

The significance of the numerous particles present on the external face of hair cell I plasma membrane, of the particle rows on the cytoplasmic face of hair cell II plasma membrane and the intramembrane organization of the efferent synapses with afferent nerve endings and fibres, are all open problems Furthermore, the existence of gap junctions (electrical synapses) between type I hair cells and the surrounding nerve chalice (Spoendlin, 1966, Smith, 1967, Hamilton, 1968), could not be ascertained in the present study

RESUME

Maculae sacculi et utriculi de l appareil vestibulaire ont été étudiés chez le chinchilla avec la méthode du cryodecapage Les empreintes montrent zonulae occludentes tres etendues qui relient les cellules de soutien entre elles et avec les cellules sensorielles du cote avoisinant l espace endolymphatique Gap junctions sont révelees entre les cellules de soutien Specialisations de la membrane cellulaire dans les regions synaptiques des deux types de cellules sensorielles sont également mise en evidence

Fig 3 Lower part of a hair cell type I (*HCI*) surrounded by its nerve chalice (*NC*) *SC* – part of a supporting cell The cleavage plane runs within the plasma membrane of the sensory cell and exposes its external face (*B*) Then it jumps within the plasma membrane of the nerve ending thus revealing its cytoplasmic face (*A*) Finally, nerve chalice and supporting cell are cross fractured Numerous large particles are seen on the external face (*B*) of the sensory cell plasma membrane more numerous particles of variable size characterize the cytoplasmic face (*A*) of the nerve chalice The external face of the outer nerve chalice plasma membrane (•) is almost devoid of particles × 44 000

Fig 4 Cytoplasmic face of the hair cell type II plasma membrane with particle rows × 58 000

ZUSAMMENFASSUNG

Die Maculae sacculi und utriculi des Vestibularapparates am Chinchilla wurden mit Hilfe der Gefrierbruchmethode untersucht In den Abdrucken stellten sich ausgedehnte *Zonulae occludentes* zwischen Sinnes und Stutz zellen an der endolymphseitigen Oberflache dar, wahrend *Gap junctions* zwischen den Stützzellen nachweisbar wa ren Einige spezielle Strukturen der Membranen im synaptischen Bereich beider Typen von Sinneszellen wurden beschrieben

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DISCUSSION

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STUDIES ON THE SENSORY HAIRS OF RECEPTOR CELLS IN THE INNER EAR

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Abstract The crista ampullaris of the semicircular canal in the frog can be isolated and mounted in a chamber so that the sensory hairs can be observed under high magnification in interference-contrast The cupula is removed and the sensory hairs can be manipulated and their mechanical properties investigated by a microprobe held in a micromanipulator The hairs appear quite stiff and pivot around their base When subjected to force they break as if they are brittle All the cilia within a bundle move together as if joined to one another Labelling for electron microscopy with polycationic ferritin reveals that the membrane surrounding the cilia has a surface coat of negatively charged molecules When the organ is incubated with polycationic ferritin before fixation the sensory hairs agglutinate Fusion of the membrane surrounding individual sensory hairs also occurs

Sensory excitation within the organs of hearing and equilibrium is triggered by the movement of the sensory hairs which project in a bundle from each of the receptor cells This movement is thought to cause deformation of some as yet unidentified structure, leading to the creation of a receptor potential The receptor potential in turn determines the state of excitation at the afferent synapse (Davis, 1965) The mechanical event taking place at the sensory hairs thus initiates the excitatory process The first part of the work presented below is aimed at investigating the mechanical properties of the sensory hairs

At the threshold of sensation the actual movement of the sensory hairs is likely to be extremely small, probably of molecular dimensions (Bekesy, 1960, Johnston & Boyle, 1967) Even so, the mode of motion of the sensory cilia must be determined by their mechanical properties and their coupling to one another These properties in turn must depend on the macromolecular organization of the core and the membranes of the cilia The second part of this report describes work dealing with these aspects of inner ear sensory cilia

Depending on what properties the sensory hairs have, several different modes of motion can be envisaged, as illustrated in Fig 1 From the zero position in the resting state, mechanical force applied to the longest of the cilia, which are the ones which are attached to the overlying structures, can cause (1) a bending of the sensory hairs if they are flexible, (2) a parallel shift if they are stiff and rigidly attached to their neighbours as well as at their base, (3) a pivoting around their base if they are stiff along their length but flexible at their base, (4) a separation of cilia if lateral coupling is weak These different modes of motion imply quite different physiological mechanisms of excitation, even if it is true that the relative scale on which these motions occur in life is grossly exaggerated in the figure

An interesting observation which focuses attention on the sensory hairs is the fact that hair cell damage leading to neurosensory hearing loss and labyrinthine dysfunction often involves damage to the sensory hairs at an early stage

An abstract of the present results has been published elsewhere (Flock & Murray, 1976)

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Fig 1 Theoretical modes of motion for sensory hairs 0, Resting position, 1, Bending of flexible sensory hairs, 2, Parallel shift when they are stiff and rigidly attached to their neighbours, 3, Pivoting around their base when they are stiff 4, Separation of sensory hairs due to weak lateral binding

MATERIAL AND METHODS

Knowledge about the mechanical behaviour of individual sensory hairs is lacking, mainly because of the inaccessibility of the sensory epithelia within the temporal bone This has been overcome in the present work by using the isolated frog crista ampullaris maintained in a chamber which permits controlled micromanipulation during visual observation The ampulla is carefully dissected free by a ventral approach and transferred to a dark-field chamber The roof and walls of the ampulla are removed with microscissors and the cupula gently detached from the crista with glass hooks In most cases this procedure damages many of the sensory hair bundles, especially along the central portion of the crista, but in successful preparations, several sensory hair bundles remain intact and undamaged Damage to sensory cells is detected by the appearance of a spherical protuberance at the base of the kinocilium, a sign of damage



Fig 2 Crista ampullaris in profile Flexible hairs due to previous fixation. Low magnification



Fig 3 At high magnification individual stereocilia can be discerned (*arrow*) × 800

familiar to electronmicroscopists After dissection the crista is held by a pair of platinum wires so that the ridge of the crista can be viewed in profile in a microscope equipped with interference contrast optics according to Nomarski The working distance of the 40 × water immersion objective is long enough (1 6 mm) to allow a microprobe to be inserted beneath the objective lens The microprobe is held by a micromanipulator (Leitz) and is used to manipulate the sensory hairs Recording could be done by micro-cinematography The prepara



Fig 4 A microprobe (*A*) approaches, and (*B*) moves an individual sensory hair bundle which is thereby made to pivot around its base × 700

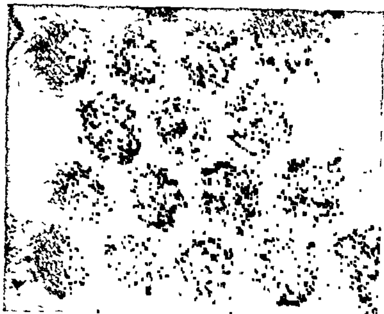


Fig. 5. Sensory hair bundles treated with Triton X-100. Cell membranes are dissolved.

tion is bathed in frog Ringer which fills the space between the chamber and the water immersion lens. With the aid of a pipette this fluid can be exchanged with test solutions and fixatives. Electron microscopy was used to study structural components of the sensory hairs.

Mechanical Properties

A low magnification view of the crista is seen in Fig. 2. At higher magnification, using the water immersion lens, the sensory hairs can be seen in considerable detail (Fig. 3). In Fig. 4A, B, a microprobe is seen to approach and move an individual sensory hair bundle. In Fig. 4B the bundle has been displaced to the right by the microprobe and it can be seen how the sensory hairs pivot stiffly around their base with the shorter stereocilia following the longer ones. This is a typical and striking feature observed during all our micromanipulations. Although the cilia are fairly free to bend at their base, they are rather stiff and brittle if subjected to bending force along their length, they tolerate only a few degrees of bending before they break at a sharp angle. This is in agreement with a remark by Engström et al. (1962), who note that when a coverglass is used to press against the unfixed organ of Corti the stereocilia tend to fan out stiffly, though it is contrary to most observations reported in the literature relating to sensory hair structure, at least in the mammalian vestibular system, such studies depicting sensory hairs as being rather flexible structures except for a portion at their base. If a solution containing glutaraldehyde is added to the chamber, the stiffness of the cilia becomes gradually reduced until micromanipulation shows that the sensory hairs now bend in a flaccid fashion. It is thus possible that the

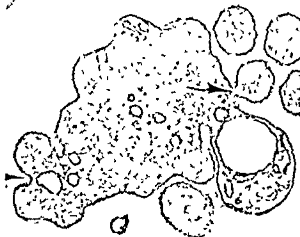


Fig. 6. Sensory hair fusion induced by the ototoxic drug Gentamicin (from Wersäll et al., 1973).

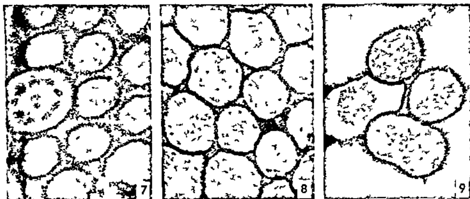


Fig 7 Sensory hairs incubated with polycationic ferritin *after* fixation with glutaraldehyde
Fig 8 Sensory hairs incubated with polycationic ferritin *before* fixation with glutaraldehyde Sensory hairs are now closer together

Fig 9 Sensory hairs incubated with polycationic ferritin *before* fixation with glutaraldehyde At this more advanced stage sensory hair fusion has occurred

arched bending seen in scanning electron microscopy of the frog crista (Hillman, 1974) is a fixation artefact It must be remembered, however, that at this preliminary stage only frog Ringer solution has been used Frog Ringer preserves the integrity of the sense organ in the isolated state but does not provide the high potassium environment to which the sensory hairs are normally exposed It cannot be excluded that the composition of the fluid bathing the hairs is of importance for their mechanical properties, a possibility which remains to be investigated The single kinocilium which is present within each bundle exceeds the stereocilia in length It is of interest to observe that even though the kinocilium is usually stiff and rigid, it can display continuous motility In at least three instances a whip-like slow spontaneous beating has been seen When this occurs, the stereocilia are moved along with the kinocilium, a fact which agrees with observations during micromanipulation and shows that the stereocilia are attached to the kinocilium This observation demonstrates the mechanical strength of the bonds between the kinocilium and the first row of the stereocilia observed in electron microscopic studies (Hillman 1974, Bagger Sjoback & Wersall, 1973 Ernstson & Smith, 1976)

The sensory hairs are covered by a membrane which is continuous with the apical membrane of the hair cell The stiffness could be due to the tubular structure of this membrane or it could be due to the properties of the core within the stereocilia Membranes of cells can be dissolved selectively, leaving proteins within cells intact, by the use of the detergent Triton X-100 When this agent is used on the crista ampullaris the membrane of the sensory hairs is dissolved (Fig 5) In preparations treated in this way the sensory hairs exhibit a mechanical behaviour and stiffness properties similar to those in the untreated organ Furthermore, the lateral coupling between the cilia of the bundle appears to be unaltered One difference is that the cuticular plate can now be seen to rock inside the hair cell as the sensory hair bundle pivots during micromanipulation These results indicate that the stiffness depends on the fibrillar core in the cilia rather than on the mem brane

Membrane Properties

Needless to say, the membrane surrounding the cilia is still of utmost importance in the functioning cell This is seen dramatically in cases of ototoxic damage to the inner ear, one example being given in Fig 6 which is from a paper by

Wersäll et al (1973) The membranes surrounding the cilia have fused, giving rise to a giant sensory hair containing the cores of previous stereocilia Apparently some property of the membrane which keeps the cilia separate has been altered by the toxic drug, giving rise to membrane fusion It is possible that this could have to do with electrostatic repulsion between charged membranes

The membrane of normal cells contain macromulecules with negatively charged side-chains which are exposed at the membrane surface The fixed negative charge is balanced by a layer of positive counter ions attracted from the surrounding medium If the membranes of neighbouring stereocilia are equipped with such charged groups, repulsive force would tend to keep them apart If the fixed negative charge of the membrane surface were reduced or neutralized, the respulsive force between the cilia would diminish or disappear and approximation and eventual fusion could in theory ensue

In order to test this hypothesis, polycationic ferritin has been used to mark negatively charged groups among the sensory hairs of the present preparation (Dannon et al, 1972) Figure 7 shows a group of stereocilia which have been incubated with cationic ferritin after fixation ith glutaraldehyde The ferritin is seen as dense particles outlining the membranes and also labelling a matrix intertwining the stereocilia If a polycation, in this case the cationic ferritin itself, is added to the chamber when the crista is unfixed, then the sensory hairs become agglutinated, the space normally separating the stereocilia being collapsed to show profiles of close packing (Fig 8) or even sensory hair fusion (Fig 9) This is reminiscent of the picture seen after ototoxic drug action (compare Fig 6)

It is also interesting to note that sensory hair fusion is seen after acoustic trauma to the organ of Corti (Engstrom & Ades, 1973, Spoendlin, 1970, Bredberg, 1973) and in a case of inherited inner ear degeneration in the guinea pig similar to hereditary deafness in man (Ernstson, 1972) As a working hypothesis, one could assume that in acoustic trauma the mechanical impact is great enough to overcome the repulsive force between the cilia and cause their fusion In the case of hereditary fusion a component of the membrane which accounts for separation by carrying a negative charge could have failed to become synthesized or to be deposited in the membrane due to genetic disorder Damage due to aminoglycoside antibiotics could be explained along the same lines Even though the similarities in experimental and pathological sensory hair fusion may be accidental and may have quite different underlying mechanisms, the possibility of a common denominator is interesting to pursue

ZUSAMMENFASSUNG

An isolierten Cristae ampullares von Fröschen wurden die Sinnesepithelien freigelegt, so daß die Sinneshaare in Interferenz Kontrast Mikroskopie studiert werden konnten Mit einer Mikroprobe wurden die mechanischen Eigenschaften der Haare untersucht Spezialmethoden für das Studium von membranfixierten Ladungen sind angewendet worden

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DISCUSSION

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THE COCHLEAR BLOOD FLOW

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Abstract Cochlear blood flow in the guinea pig was investigated using Tracer Sephadex® 15 microspheres labelled with radioactive nuclides A reference organ technique was used for quantitative measurements of cardiac output and the blood flow in the cochlear soft tissue, CNS and kidney In some experiments the blood flow was measured at two occasions, either with ^{57}Co- or with ^{58}Co labelled microspheres The results, including the size of cochlear blood flow are presented and discussed

The Cochlear Blood Flow

There are several different fluids in the cochlea Besides endolymph and perilymph there is the blood, which is in close relation to the other fluids and on which they are dependent in many respects Evidence is accumulating that the blood in the inner ear is the origin of the endo- and perilymph (Schmieder, 1973, 1974*a* Kellerhals, 1974) and that many inner ear disorders are caused by pathologic changes in the blood vessels (Hawkins, 1973, Johnson, 1973) In all, this prompts further studies regarding the inner ear blood flow

There are several ways of examining the blood flow in a peripheral organ Most of these methods are not applicable to the inner ear with its delicate structures hidden in the bone Some methods, such as direct observation (Yoshioka, 1957, Perlman & Kimura, 1962, Costa & Brånemark, 1970*a*, 1970*b* Matsuyama, 1970, Lawrence, 1970, Lawrence & Clapper, 1972) or impedance plethysmography (Morimitsu et al, 1965, Suga & Snow, 1969, Suga, 1970, Snow & Suga, 1974) enable qualitative information to be obtained on the cochlear blood circulation However, the magnitude of the inner ear blood circulation has long been unknown Recently Clairmont et al (1973), Jackson et al (1974) and Pollock et al (1974), investigated the blood flow in the entire temporal bone, utilizing the microsphere technique As the blood flow to the bony walls and to the membranous labyrinths is derived from different vessels (the external carotids and the vertebral arteries) it is important that the membranous parts are studied separately

In this study the microsphere method was adopted in order to estimate the magnitude of the cochlear blood flow The results indicate that the method is suitable for estimating changes in blood flow and for evaluating the effects of various pharmacological agents routinely used in clinical practice

This work has been sponsored by the Swedish Medical Research Council and Tysta Skolan (Project No 04782)

MATERIALS AND METHODS

The experiments were performed on pigmented young, healthy guinea pigs, weighing 250–500 g, with normal middle ears and with normal Preyer reflexes Anaesthesia was induced by injection of Inactin® (Chem Fabrik Promonta GmbH, Hamburg, West Germany) (60 mg/kg b w) i p Constant body temperature was secured through a servo-controlled heating pad After tracheostomy, the right common carotid was catheterized and connected to a pressure transducer Replacement of fluid losses was effected through a catheter in the right jugular vein A catheter

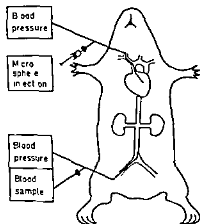


Fig 1 A diagrammatic drawing of the experimental set up

was inserted in the left iliac artery and advanced into the abdominal aorta. This catheter was connected to a pressure transducer and a pump. After recovery from surgery (about 30 min), the carotid catheter was inserted into the left ventricle of the heart of the still anaesthetized animal. A successful catheterization was signalled by an increased pulse height as visualized on a recorder. Free passage in the iliac catheter was indicated by blood pressure recordings that were not significantly influenced by aspiration or infusion of blood at a rate of 0.15 ml/min. The complete experimental set up is illustrated in Fig. 1. For the measurements of blood flow the microsphere method (Rudolph & Heyman, 1967, Wagner et al., 1969) was used, utilizing radioactive dextran microspheres (Tracer Sephadex® Pharmacia Fine Chemicals, Uppsala, Sweden). The density of the particles is close to that of red blood cells (1.12 ± 0.02 S.D. g/ml). The particles can easily be labelled with radionuclear isotopes according to the specific experimental requirements. The size distribution of the microspheres is even (15 μm $\pm$ 3 μm S.D. or 50 μm $\pm$ 6 μm S.D.). In general, each microsphere was labelled to give a counting rate of a few counts per minute. This was done to avoid excessive radioactivity. The radionuclides used were ^{57}Co with peak gamma energy of 0.122 MeV and ^{58}Co with peak energies of 0.511 MeV and 0.810 MeV. The energy peaks of ^{58}Co overlap the energy peak of ^{57}Co (about 30%). This disadvantage was overcome by lower specific labelling with ^{58}Co. As a rule, the specific activity for ^{58}Co was about 20% of that of ^{57}Co, thus giving a net crossover of some 5%.

Injection of microspheres

A one ml plastic syringe was heated over a flame and drawn out into a catheter-like tip. 0.1 ml of saline containing 500 units/ml Heparin and an adequate number of suspended microspheres (15 μm microspheres approximately 2×10^4, 50 μm microspheres approximately 2×10^3) were filled into the syringe. After connecting the syringe to the ventricular catheter about 0.3 ml of blood was permitted to flow into the syringe. The spheres–saline–blood was slowly mixed by means of a plastic-coated piece of iron inside the syringe and an externally placed magnet. Just prior to slow injection, the pump was started in order to withdraw blood at a constant speed (0.15 ml/min) from the abdominal aorta. The withdrawal was continued until 10 seconds after the end of the injection. This procedure could be repeated a second time utilizing microspheres labelled with another isotope. Pilot studies were performed when the microspheres were injected without prior mixing with blood. The results obtained, however, showed considerable variance in the number of trapped microspheres. It was also noticed that the mere observation of blood flowing into the sampling catheter was insufficient for the detection of flow restrictions. The simultaneous recording of the abdominal blood pressure eliminated false results of this origin. The cochlea was fixed in 2.5% glutaraldehyde to ensure stability of the binding between the radionuclide and the microspheres. Microdissection of the cochlea was performed. The bony parts were removed and the radioactivity in the soft parts including the nerve tissue in the modiolus was counted in a gamma spectrometer. Values for cochleas with less than 30 spheres (N) trapped were discarded from the results due to a high error of randomness [percent error of randomness $= (\sqrt{N}/N) \cdot 100$]

those of the bony walls cannot be excluded In such cases there is a considerable risk that the blood flow changes in the soft tissue would not be detected

In the procedure used in these experiments the right common carotid was ligated without influencing the blood flow in the right membranous cochlea in comparison with that in the left This is probably because the blood flow in the bony parts of the cochlea is mainly derived from the external carotid artery whereas the membranous inner ear gets its blood supply from the basilar artery (Anson et al, 1966)

By choosing the young guinea pig in which the cochlea is relatively easy to dissect without using burrs, it was possible to get the soft parts of the cochlea separated from the bone without contaminating the surroundings

Blood circulation studies with radioactive microspheres are relatively easy and reliable in organs with a high blood flow For statistical reasons a minimal number of spheres must be trapped in an organ to minimize random variations As the blood flow in the cochlea represents about 1/10 000 of the cardiac output, at least one million spheres must be injected into the heart This is a relatively large number and the spheres will occlude a certain number of capillaries For valid conclusions to be drawn after a second injection of spheres, it is essential that this capillary occlusion does not interfere with the normal central or peripheral circulation

The magnitude of the blood circulation in the cochlea was determined with Tracer Sephadex® 15 It was found possible to make two consecutive determinations of the blood flow without systematically disturbing the cardiac output, the blood pressure, the blood acid-base balance or the rate of the blood flow in peripheral organs Thus, it is reasonable that a change in the blood flow, determined by this method, will reflect a change caused by manipulations within the interval between the two injections of microspheres

Investigations with the microsphere method are being continued to study how manipulations with the cochlea may change the inner ear blood flow and several other questions concerning the reactions of the inner ear blood circulation may also be answered Some investigations have already indicated that noise will induce changes in the blood flow leading to loss of hearing thus noise has been demonstrated to change the perilymph turnover (Schnieder, 1974b Kellerhals, 1974), to change the blood flow in the vessels (Lawrence et al, 1967, Perlman & Kimura, 1962) and to change thrombocyte adhesion (Maas et al, 1973, Maas & Keller, 1974) With the microsphere method it might be possible to conduct further investigations into the effects of noise on the cochlear blood flow

Several drugs have been used to improve the inner ear blood flow One example is nicotinic acid in Meniere's disease and another is dextran in sudden deafness or after exposure to noise (Kellerhals et al, 1971) There is no evidence that these drugs really influence the blood circulation of the inner ear and it is essential to develop a method to test the effect of drugs on the inner ear blood flow In this respect the microsphere method is very promising

ZUSAMMENFASSUNG

Bei Meerschweinchen wurde der Blutstrom der Cochlea mit radioaktivgezeichneten Tracer Sephadex® 15 Mikrosphären untersucht Fur quantitative Messungen der CO und des Blutstroms in den Weichteilen der Cochleas CNS und den Nieren benutzte man Referenzorgantechnik Bei einigen Experimenten hat man den Blutstrom zweimal mit entweder ^{57}Co oder ^{58}Co-gezeichneten Mikrospharen gemessen Die Resultate d e die Große des Blutstroms in der Cochlea einbegre fen werden präsentiert und diskutiert

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DISCUSSION

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THE DELAYED EFFECTS OF ETHACRYNIC ACID ON THE STRIA VASCULARIS OF THE GUINEA PIG

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Abstract Guinea pigs were administered 40 mg ethacrynic acid per kg b wt and sacrificed at 30–48 minutes 3–4 hours 2 or 7 days post-drug Cochlear potentials (EP and CP) were monitored before sacrifice At 30–48 minutes the potentials had decreased considerably, and a marked edema plus cytological changes were visible in the stria vascularis The potentials had recovered to about 75% of their original value at 3–4 hours some cell recovery was visible, but the edema was still present Potentials recorded from the basal turn were normal at 2 and 7 days although some strial cells showed deterioration

Within the past 10 years, there have been a number of cases reported (Schneider & Becker, 1966, Meriwether et al, 1971) where transient or permanent hearing loss occurred in patients receiving the diuretic drug, ethacrynic acid Quick & Duvall's study in 1970 revealed that the Preyer reflex was lost within 5 minutes after injection of doses of 50 mg/kg or more in guinea pigs Electron microscopic examination revealed there was a marked edema in the stria vascularis in animals sacrificed at 10 minutes to 4 hours after injection Bosher et al (1973) found that both the a c cochlear potential and the d c endocochlear potential dropped dramatically shortly after the drug was administered to rats They likewise reported edema and ultrastructural changes in the stria vascularis 2 hours after the ethacrynic acid was given

West et al (1973) found that the a c cochlear potential (CP) followed a well defined time course in guinea pigs which had received 40 mg/kg of ethacrynic acid intravenously Within 20 min, the voltage dropped to 1/5th of its original value, it then recovered slowly over a period of 3–5 hours Apparently some changes were occurring within the cochlear duct which were responsible for these electrical alterations We believed that electron microscopic studies correlated with measurements of the electrical potentials might give us some insight into the basic phenomena involved In this paper, we report the ultrastructural changes found in the stria vascularis correlated with measurements of CP and the d c endocochlear potential (EP) from 30 min to one week after drug administration in guinea pigs

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MATERIALS AND METHOD

Fifteen young, healthy guinea pigs with active Preyer reflexes, were used in the experiments

Electrophysiological techniques

The sound stimuli were generated using a model 555 Western Electric speaker, and were conducted to the external auditory meatus through a metal tipped rubber tube which was fitted snugly into an ear speculum sealed into the meatus of the left ear The CP was monitored with a small silver ball electrode placed on the round window membrane

The electrical potential was amplified 1 000 × with a Keithley differential amplifier and measured on a General Radio wave analyser (Model 1900a) After the electrophysiological meas

ures were completed, the sound system was calibrated by measuring the sound produced just lateral to the tympanic membrane by a probe tube attached to a calibrated microphone inserted into an ear speculum Details as to how the ossicles were kept free of accumulated fluids, the body temperature maintained, the sound generated, radiation artifacts prevented and the sound measured have been described previously (Vernon et al, 1976)

The EP was monitored by means of a silver/silver chloride wire electrode, inserted into a glass micropipette filled with KCl (140 mEq/l and NaCl, 2 mEq/l, Bosher & Warren, 1968) The tip of the pipette was drawn to be approximately 1 μm inside diameter It was inserted through the intact round window and basilar membranes into the cochlear duct by means of a micromanipulator The d c potential was monitored on a Tektronix oscilloscope

Morphological techniques

All animals were fixed with Dalton s 1% osmium tetroxide while they were still anesthetized Animals 541, and 613 were taken off the respirator (and given artificial respiration when necessary) before fixation All the other animals were left on the respirator

The labyrinthine perfusion was accomplished by our standard techniques (Smith & Dempsey, 1957) The left ear of each animal was perfused for a total of 3–5 min over a period of 10–15 min The animal was then decapitated and the temporal bone removed The cochlea was opened at the apex and round window, re-perfused briefly by dripping the fixative through the apex and immersed in the OsO_4 for a total time of 1½ hours Most of the otic capsule was removed during dehydration in 70% ethanol Any pieces of the membranous labyrinth that fell off during dissection were retrieved and embedded separately, the rest of the cochlear duct still attached to the bone was embedded in one piece All were embedded in araldite, with toluene as an intermediary agent to promote diffusion of the epoxy resin

One micron sections were made and stained with toluidine blue Thin sections were stained with uranyl acetate and lead citrate for electron microscopy Samplings were taken at 8–12 different locations from basal to apical ends in each cochlea

Experimental techniques

Two different procedural techniques were used when preparing the guinea pigs for the electrophysiological measures The first procedure was used for the animals to be fixed within 4 hours after the administration of ethacrynic acid In these animals anesthesia was induced by the i p injection of Dial (60 mg/kg) with urethane (240 mg/kg) An indwelling polyethylene (PE50) catheter was secured into the left jugular vein and kept full of heparinized saline to prevent clotting A tracheotomy tube was inserted and the animal was mechanically respirated with room air using a Harvard small animal respirator The rate and depth of respiration were adjusted so as to prevent spontaneous contractions of the middle ear muscles The round window of the cochlea was exposed, the animal connected to the sound generator and the round window electrode put in place The amount of sound required to produce one microvolt of CP at fifteen different frequencies over a 0 1 to 20 kHz range was determined This is referred to as the 1 μV isopotential function

A 1 kHz tone was then introduced into the ear at an intensity to produce 150 μV CP (The intensity of the sound was approximately 1 dyne/cm^2) The CP was monitored for 5–10 min before the experimental drug was given

The ethacrynic acid (40 mg/kg) was introduced through the jugular catheter, over a 1 min period The injection solution contained 10 mg/ml of ethacrynic acid, and had an osmolarity of 143 mosmols as measured by an Advanced osmometer The CP was continuously monitored with readings taken at least every 1–2 min for the first hour, and then every 5 minutes for 3–4 hours for animals 613 and 650 Animals in which the EP was measured had the d c potential continuously monitored by observation on the oscilloscope screen

A slightly different schedule was used for the chronic experiments These guinea pigs were initially anesthetized with pentobarbital (35 mg/kg) administered i p and they were *not* tracheotomized Otherwise they were prepared in the same manner as those just described After the 1 μV isopotential function was completed, sufficient sound at 1 kHz was introduced to the ear to produce approximately 150 μV of CP The single dose of ethacrynic acid (40 mg/kg) was given i v and the CP monitored until it began to recover from its lowest level After it was ascertained that the ethacrynic acid had indeed produced its usual effect upon the CP, the electrode and ear speculum were removed, *the incision closed with wound clips, and the* animal allowed to recover from the anesthetic At the chosen period (48 hours or 1 week post-drug), the animal was given Dial with urethane for its second anesthetic, and prepared according to the first schedule described above

The experimental animals were fixed at the following times after ethacrynic acid administration

Animal no

541, 30 min post drug CP monitored
695, 48 min post drug, EP monitored
[illegible]), 208 min post drug, CP monitored
[illegible], 230 min post drug, CP monitored
?696, 48 hours post drug, CP and EP monitored
700, 48 hours post drug CP and EP monitored
703, 49 hours post drug CP and EP monitored
697, 1 week post drug, CP and EP monitored
699, 1 week post drug CP and EP monitored
704, 1 week post drug, CP and EP monitored

Four controls were prepared Guinea pig 694 was a fixative control only The other three were given a 0 07 M sodium chloride solution (NaCl) i v (which is iso osmolar with the ethacrynic acid) in the same amount as the drug, and were surgical controls Animal 706 was fixed at 34 min after the NaCl was given, the electrical potentials were not measured Animal 644 was fixed approximately 17 minutes post NaCl and the a c cochlear potentials measured Animal 698, fixed 3 hours post NaCl, had the EP continuously monitored throughout that period

RESULTS

Control Ears

The 1 μm sections as well as the electron micrographs of the control ears revealed a cell architecture and cytological structure (Fig 1*a*) which were similar to those previously described (Smith 1957, Hinojosa & Rodriguez Echandia, 1966) Animal no 706 showed a generalized deterioration of the stria in the basal turn, which appeared to be of long duration and not due to surgery No other control animals showed pathology

The free apical surfaces of the marginal cells from animals 644 and 698 showed a thick surface coat (Fig 2*a*) Strands of the same dense material extended into the endolymph The coat was thicker in the surface crypts, but did not extend down into the junctional complexes between the cells The surface coats of the stria vasculares from animals 694 and 706 were negligible, and only obvious in the crypts

The apical cytoplasm of the marginal cells (Fig 2*a*) was characterized by the following components (1) moderate numbers of round or oval mitochondria, (2) many free ribosomes, (3) some Golgi apparatus with dilated cisternae (4) some rough endoplasmic reticulum (large amounts in some cells), and (5) many vesicles *and cisternae of variable size The vesicles* were lined with a material similar in thickness to that of the surface coat but of less density The lining was approximately 150 Å in thickness, whether the vesicles were intermediate in size or small so that the lumina of the smaller ones were completely filled with the material These vesicles may have been derived from coated vesicles which were also present but no outside coating was visible on the larger ones, and we have termed them "lined vesicles"

The basal two thirds of the marginal cells consisted of many finger like processes, which were formed by infoldings of the plasma membrane These processes were fairly straight, although rugosities were present on those close

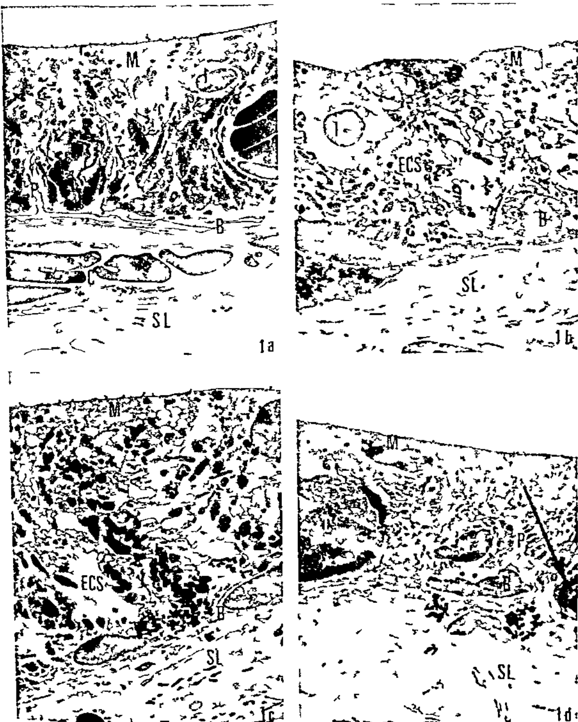


Fig. 1. (*a*) Stria vascularis from animal 644, surgical control, second turn. × 3 650. (*b*) Stria vascularis from animal 695, 48 minutes post-drug, third turn. × 3 460. (*c*) Stria vascularis from animal 613, 730 minutes post drug, basal turn. × 3 660. (*d*) Stria vascularis from animal 696, 48 hours post-drug, third turn. Note evidence for degeneration at right (*arrow*) and capillary packed with erythrocytes and concentrated plasma protein at left (*C*). × 3 700. *B*, basal cells; *C*, capillary; *ECS*, extracellular space; *I*, intermediate cell; *M*, marginal cell; *P*, basal processes of marginal cell; *SL*, spiral ligament.





Fig 4 (*a*) Ap cal end of marg nal cell from no 696 48 hours post-drug, showing granularity of cytoplasm ×60 000 (*b*) Basal process of marg nal cell from no 700 48 hours post-drug showing accumulation of dense mater al in extra cellular space (*wl te arrows*) in p n cyt c ves cles (*black arrows*) and n ntracellular bod (×) ×50 000 *Mi* m tochondr on *S* surface coat

cesses maintained their position adjacent to the capillaries but the basal lamina no longer filled ie interspace between endothelium and pro es It was condensed and retracted from the ter endothelial wall

Animal 695 was fixed 48 min post drug The EP had been monitored for 29 min A potential of 86 mV was recorded initially This had fallen to its lowest point of 16 mV at 22 min and had reversed its downward course to 10 by 29 min when the micropipette was withdrawn and preparation for fixation begun (Fig 3)

This ear also demonstrated edema (Fig 1*b*) but it was not as marked as in no 541 For example adjacent marginal cell membranes were apposed for some distance below the junc tional complexes and mitochondrial changes were less A thin surface coat poorly stained was visible on the marginal cells (Fig 2*b*) The capillary basal lamina had the same constricted appearance as in no 541

208 and 230 minutes after ethacrynic acid

The CP voltage at 1 kHz for no 650 was meas ured originally at 165 μV This had dropped t a low of 41 μV 14 min post drug and recovere slowly over a 3 hour period to 120 μV at 19 min The animal was fixed at 208 m n post dru (Fig 5)

The CP for no 613 was 153 μV at the time of drug administration It dropped to a low of 35 μV at 16 minutes and recovered slowly to 113 μV at 3 hours The ear was fixed at 230 min post drug

The stria vasculares from these 2 animals showed rather similar alterations Edema was present in both left ears (Fig 1*c*) but the mar ginal cells were flat not bulging into the endo- lymph There were well defined surface coats on many marginal cells The extra cellular fluid collection was greater in no 650 s ear some marginal cells were separated up to the junc- tional complexes In no 613 the cell membranes

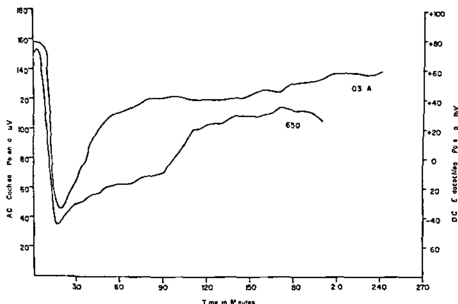


Fig 5 Graph showing the cochlear potential curve for 1 kHz for no 650 fixed at 208 min post drug This is compared with the EP from an animal from another series (Brummett et al unpublished data) which was treated in the same manner as no 650 but had its EP monitored The decline and recovery of the two curves are similar

were apposed well below the luminal surfaces and interdigitating processes even present The apical cytoplasm from many of the marginal cells looked much like the controls, with numerous lined vesicles (Fig 2*c*) Animal 650 had many cells with the small vesicles and cisternae described at 30 min post drug

Some marginal cell features were common to both animals There were many large apical cisternae containing dispersed debris and many membrane bound structures filled with vesicles, debris and osmiophilic material (Fig 2*d*) The basal cell processes had partly reverted to normal The intermediate cells appeared more shrunken than at 30 min, although there were no obvious changes in organelles

The endothelium of the strial capillaries seemed thicker, and the ratio of micropinocytic vesicles on the outer surface relative to those at the luminal surface was about double that of

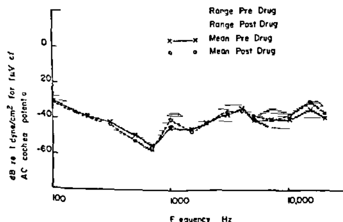


Fig 6 Graph showing means and ranges of the 1 μV isopotential function taken before drug administration (pre-drug) and again just before sacrifice (post-drug) from all animals fixed at 48 hours and one week.



Fig 7 Stria vascularis from no 697, one week post-drug, middle second turn, showing a degenerating intermediate cell (*I*) at extreme right, and a collected mass of cell debris at lower left (*arrow*) ×5 600

the controls or at 30–48 minutes The basal lamina was still compressed

Two days after ethacrynic acid

The three animals sacrificed at 48–49 hours post-drug had normal CP and EP measurements just before fixation (Fig 6) although the CP monitored immediately after ethacrynic acid administration had demonstrated the usual 120–130 μV loss within the initial 15–20 min

No abnormal accumulation of extracellular fluid was seen in the stria vasculares of any of the three ears (Fig 1*d*) However, another phenomenon had made its appearance vascular stasis of the strial capillaries This varied from place to place within the stria vascularis and among animals Some capillaries were packed with erythrocytes, but the plasma was normal in appearance The plasma protein was so dense in other capillaries that it was difficult to distinguish between erythrocytes and plasma In the latter case, the capillary outlines were irregular in shape, the endothelial nuclei distorted and the luminal endothelial wall defined with difficulty The basal lamina about the endothelial cells had its normal dispersed form

Many of the marginal cells had a quite granular cytoplasm (Fig 4*a*) and ill defined intracellular membranes, basal cell processes were of normal size A dense surface coat was visible

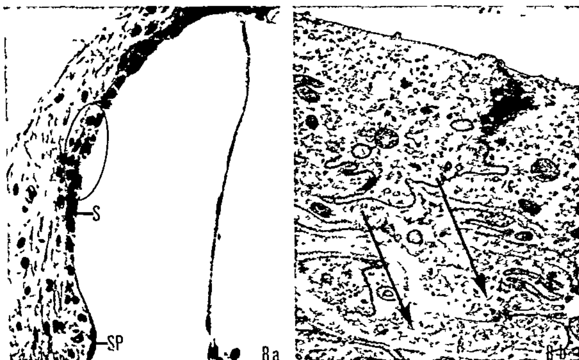


Fig 8 (*a*) Light micrograph of atrophic stria (*S*) and normal spiral prominence (*SP*) from no 699 one week post-drug lower third turn ×500 (*b*) Electron micrograph of cell in region encircled in (*a*) showing intercellular debris (*arrows*) ×19 000

on some marginal cells (Fig 4*a*) it was very thin and poorly stained on others A granular material of moderate density was frequently present in the extracellular space between marginal and intermediate cell processes and filled the pinocytic vesicles of the latter cells (Fig 4*b*) Membrane bound bodies (Fig 4*b*) filled with a similar material were often seen in isolated cell processes which seemed to be mostly intermediate cell processes

There appeared to be evidence for cell degeneration in the presence of some scattered cell debris, as well as occasional cell processes which were almost devoid of cytonet or organelles

One week after ethacrynic acid

The 3 animals which were sacrificed at one week post drug (nos 697 699 704) demonstrated normal CP and EP just before sacrifice (Fig 6) No edema was present in the stria vascularis of no 697 or of 704 one sample in the second turn of no 699 showed a mild edema

The stria vascularis from no 697 revealed vascular stasis in some places capillaries were packed with erythrocytes and dense plasma protein In other places, the capillaries were normal in appearance There was some evidence for degeneration of the intermediate cells (Fig 7), there was almost no cytoret in some cells, whereas adjacent cells had quite dense cytoplasm This latter was characteristic of many intermediate cells in this animal They were packed with much RNA and rough endoplasmic reticulum and only their location aided in their identification There was degeneration in some marginal cells at the tympanic edge of the stria in the lower half of the fourth turn Otherwise the major difference in the marginal cells between this animal (no 697) and the controls was that the basal processes seemed thicker and fewer in number than normal and their cell membranes were smooth and without rugosities

Animal 699, on the other hand, showed a complete loss of the differentiated marginal and

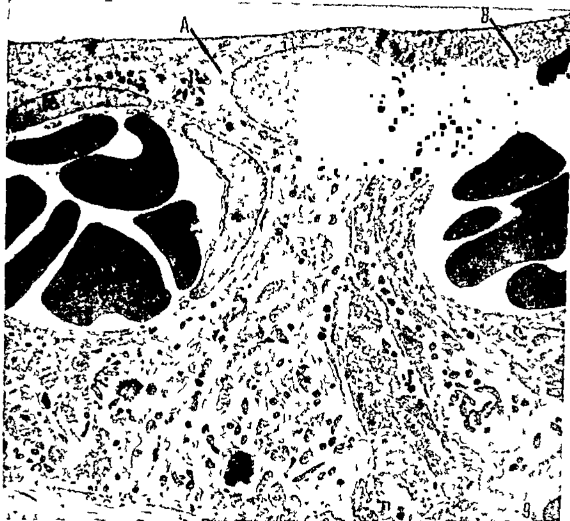


Fig 9 Stria vascularis from no 699 lower second turn one week post-drug illustrating the variability in marginal cell recovery Cell A (*center*) is normal in appearance Cell B (*right*) shows marked cytoplasmic alterations ×5 600

intermediate cells above the middle of the second turn (Fig 8*a* *b*) A non specialized cuboidal epithelium covered several layers of basal cells Some granular intercellular material of moderate density was present in some places This had the appearance of remnants of basal lamina Collections of degenerative debris were also visible Spiral prominence cells were present and normal in appearance The stria below the second turn was fairly normal in appearance There was no evidence for capillary stasis in the samples examined

There was cell degeneration in the middle first turn of no 704 A granular material was present in the extracellular space in many samples from base to apex

The stria vasculares of these one week animals showed a considerable variability both within each cochlea as well as between animals Some marginal cells seemed quite normal whereas adjacent cells appeared to be in a state of degeneration (Fig 9) This was also true of the intermediate cells More studies are needed to determine if the above variations are remarkably greater than those found in normal animals

DISCUSSION

The times chosen for fixation of the cochleas were arranged to coincide with specific changes in the cochlear potentials The two animals in the first group were fixed as soon as there was evidence that the potentials were recovering from their initial drop, and the type of alterations in their stria vasculares was essentially the same The most prominent change was a marked accumulation of extracellular fluid A similar edema was found by Quick & Duvall (1970) in guinea pigs sacrificed at 10 min and at 5 hours post-drug, and in rats (Bosher et al, 1973) at 2 hours post drug Edema in the stria vascularis thus seems to be a well-documented effect of ethacrynic acid administration at high concentration Our studies have demonstrated in addition that there are cytoplasmic changes in the marginal cells (replacement of the large lined vesicles with dense ovoid structures, mitochondrial alterations and loss of cell surface coat) There was shrinkage of the marginal, intermediate and basal cells, but no obvious alterations in the organelles of the latter two cell types

The 2 animals fixed between 3 and 4 hours were chosen to coincide with the time at which the electrical potentials had almost recovered to their original values The edema was still present, but apparently the total accumulation of fluid was less, because the cells no longer bulged into the endolymph space The intermediate cells seemed more constricted than at 30 min A prominent cell coat was visible on many of the marginal cell surfaces, and most of the lined vesicles and mitochondria examined were apparently normal There was evidence for some degeneration within the marginal cells in the presence of multivesicular bodies and many large membrane-bound bodies containing osmiophilic material Probably some mitochondria or other structures had been damaged beyond repair and incorporated into secondary lysosomes Previous experiments (Bosher et al, 1973, Brummett, unpublished) have indicated that the EP and CP recovery curves closely approximate each other and it seems reasonable to assume that the EPs of no 613 and no 650 had a recovery period similar to that recorded for the CP The structure of the apical ends of the marginal cells in nos 613 and 650 appeared fairly normal and it might be inferred that the EP is more closely related to the condition of the apical ends of the marginal cells, than to that of the basal processes or to the intermediate cells Nevertheless, at 48 hours, when EP had normal values in 3 animals, the apical ends of many cells did not have normal appearing lined vesicles, and the surface coats were thin It may be that the enzyme system originally inactivated by the ethacrynic acid was "75%" restored at 3–4 hours, but had not completely overcome the process responsible for the edema, so that precise correlations with morphology at this period are not possible It may also be that a full complement of active cells is not necessary to produce what we consider to be a "normal" EP Some further experimental work is necessary to clarify this paradox

The lined vesicles which are so prominent in the normal marginal cells are probably pinocytic vesicles Hinojosa (1972) demonstrated that ferritin was present in similar vesicles after tracer injection into the endolymph, although he called them "coated" vesicles Most of the lined vesicles are larger than coated vesicles and have no visible external coating If coated vesicles expanded after budding off from the cell membrane, the coating could be so dispersed as to be indetectable In that case, the inner lining should also become thinner, but actually its width was almost the same in all sizes of lined vesicles An alternate explanation for the size of the larger vesicles could be that they are the results of fusion of smaller endosomes, and that the outer membrane was altered during fusion A fair number of small coated vesicles can be identified but they are generally found in the interior of the cell close to the Golgi apparatus Steinman et al (1976) have recently studied pinocytosis in macrophages and fibroblasts and claimed that pinocytic vesicles were not coated at all but that coated vesicles in the same cells were derived from the Golgi and in-

volved in membrane recycling This hypothesis would fit our observations on the marginal cells The frequent crypts and invaginations at the marginal cell surface are filled with the osmiophilic surface material If such are indeed the origin of the lined vesicles, then the lining must change as soon as the vesicle is interiorized, for the linings do not have the electron density of the thick surface coat in normal cells At present the significance of the dense surface coat is unclear Only a thin surface coating was seen in some of the controls At 2 and 7 days post EA its presence was erratic Rao et al (1972) have observed that the staining qualities of surface coats are quite variable even from cell to cell in the intestine Whether the irregularity in the stria vascularis reflects a functional change, a normal variation or a staining artifact is not clear at present

It seems probable that the small ovoid structures filled with dense material found at 30–48 min post-drug are actually collapsed lined vesicles When cell water is drawn from the marginal cells to the extracellular space, the cytoplasm would become more condensed and water would be withdrawn from the vesicles inducing collapse When the process is reverted and water returns to the cells, the vesicles would readily regain their normal shape and appearance, which they indeed have 3–4 hours later

It is generally conceded that ethacrynic acid interferes with sodium and chloride transport in the kidney tubules, which causes an increased urine excretion The extracellular fluid accumulation produced by the drug in the stria vascularis may be the result of a comparable process Both marginal and intermediate cells appear to be shrunken, and there seems to have been a gross fluid movement from inside to the outside of the cells There does not appear to be any great amount of precipitable protein in the extracellular fluid within a 4-hour period so we might assume the fluid has been made hypertonic by ions either pumped out of the cells or accumulated in some other manner Water movement from within to outside the cell would naturally follow A hypertonic extracellular fluid could also draw fluid out of the capillaries and this could explain the bulging of the marginal cell surfaces into the endolymph Thalmann (1975) has recently postulated that adenylate cyclase may be the drug target in the cochlea Whatever system is attacked by the ethacrynic acid, the primary action of the drug appears to be temporary and reversible, because the edema disappears and the electrical potentials have normal values 48 hours later

Nevertheless, some cells displayed abnormal features at the one week period Many marginal cells seemed to have a reduced number of basal processes Marginal cells which seemed to be degenerating were found scattered throughout the cochleas, more often in the apical turns Accumulations of cell debris were also seen There was obvious degeneration of some intermediate cells but still an abundance of cells in the intermediate location We had no way of knowing if these cells had migrated from elsewhere (the basal cell layer, perhaps), or if they were new cells No mitosis was ever seen Despite these alterations, the cells were active enough to produce a normal lower basal turn EP A normal CP was measured at the round window

A long segment of completely degenerated stria vascularis was found in one animal (no 699) and there appeared to be some degeneration in the other two one-week animals in the fourth turn It is possible that the degeneration in no 699 was not drug related However, it did not appear to be a defect in embryological development, because the basal cell layer was present below the cuboidal epithelium, a fully differential spiral prominence epithelium was in its usual position, and some intercellular debris was visible between the cuboidal epithelial cells This finding does serve to emphasize that EP measurements are quite local monitors, for the lower basal turn EP in this animal was of normal value It is possible that cell loss of the extent found in no 699 and the degeneration seen in other animals at 2 and 7 days were not primary effects of the drug, but secondary effects of the vascular stasis and consequent hypoxia

The longitudinal extent of the capillary stasis was not determined, but it was much more prevalent than in any of the controls or experimental ears from animals sacrificed before 5 hours and at one week Furthermore, endothelial changes were associated with the vascular congestion The excessive extracellular hydrostatic pressure may have resulted in constriction of some of the sphincters on either side of the capillary bed If the capillaries are inactive long enough to damage the endothelial cells, their permeability changes and excess plasma protein can escape Permeability changes can occur within a few minutes (Milnor, 1968) This could have been the source of the dense material found in the extra-cellular space as well as in intermediate cell pinosomes Kimura (1973) has shown in experimental studies that alterations in the marginal cells of the stria as well as changes in the organ of Corti followed venous blockage for periods as short as 15 minutes The vascular stasis in this series was confined to the stria vascularis and at 2 days was quite widespread within that structure It is possible that if the stasis persists, cell degeneration will follow

Finally, the changes described are probably dose-related However, comparable cellular changes might be present in patients who have experienced temporary hearing loss, as these patients have usually suffered renal impairment (Schneider & Becker, 1966, Meriwether et al, 1971) a condition in which blood clearance (and consequently drug clearance) is quite inadequate Crifo (1973) found that guinea pigs receiving 1 mg/kg of ethacrynic acid for periods of up to 10 days had hearing deficits as well as morphological changes Further studies are needed to clarify the potential ototoxicity of this as well as more commonly used diuretics

RÉSUMÉ

Ethacrynic acid fut donné à des cobayes par voie intraveneuse, les changements des potentiels électriques cochléaires (CM et EP) furent comparés avec les résultats de l'examen électron microscopique Les deux potentiels tombaient bientôt après que la drogue avait été donnée et remontaient lentement dans une période de quatre heures Les cobayes, tués après trentes minutes, montraient l'oedeme dans la stria vascularis

ZUSAMMENFASSUNG

Nach i v Injektion von Etacrynsaure bei Meerschweinchen wurden die Änderungen des Wechselstrompotentiales der Schnecke und des endolymphatischen Gleichstrompotentiales mit elektronenmikroskopischen Befunden verglichen Beide Potentiale sanken kurz nach der Injektion auf niedrige Werte ab und erholten sich langsam wahrend der nachsten 4 Stunden Tiere, die nach 30 Minuten getotet wurden, zeigten außer Änderungen im Zellplasma Verlust des Zelloberflachenüberzuges und starkes Ödem der Stria vascularis Vier Stunden nach der Arzneimittelinjektion zeigte die Stria immer noch starkes Ödem und Zellveränderungen, obwohl beide Potentiale auf ungefähr 90% ihrer ursprünglichen Werte zuruckgekehrt waren

NOTE ADDED IN PROOF

Subsequent studies on variations in the stria vascularis in normal guinea pigs by use of serial one-micron sections indicate that there is considerable cellular variation, especially in the fourth turn Although we have not found to date any animal with the cellular arrangement below the third turn as found in animal no 699 from the present series, we belive the "atrophy" seen in no 699 is probably not the result of ethacrynic acid action

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DISCUSSION

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MODERN METHODS FOR MEASUREMENT OF BASILAR MEMBRANE DISPLACEMENTS

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Abstract Basilar membrane displacements in response to sound at threshold intensities are in the fractional Ångstrom range Visual measurements as used by Békésy in his pioneering studies are by definition limited to values above 10 000 Å The present paper discusses a number of modern techniques capable of taking measurements at lower Ångstrom levels One point methods (capacitive probe Mossbauer effect laser interferometry and optical heterodyne spectroscopy) and pattern-assessing methods (time averaged and real time holography) Advantages and disadvantages of these methods are being discussed

The acoustic energy that is entering the ear when it receives sound cannot be assessed directly The parameter that is most easily measured is the *displacement amplitude* of a given structure for a *specified sound pressure level* (*SPL*) When the first absolute measurements of vibratory displacement amplitudes of the tympanic membrane and the basilar membrane were obtained they turned out to be extremely small This is another way of saying that the ear is an exceedingly sensitive detector of acoustic energy According to a basic axiom of Information Theory, information transmitting systems while carrying out their functions expend very small amounts of energy—and the ear is no exception in this respect

Fig 1 shows displacement amplitudes of the tympanic membrane (Wilska 1935) and of the basilar membrane (Bekesy 1947) at the human auditory threshold Entered in the same graph for ready comparison are selected magnitudes of atomic and sub-atomic structures and/or events Amplitudes of 10^{-9} cm (≈0 1 Å) of the tympanic membrane and that of 10^{-11} cm (≈0 001 Å) of the basilar membrane are indeed extremely small, difficult to grasp for anyone

It is important to realize that in 1947 when Bekesy performed these measurements such small magnitudes could not be measured directly Visual assessment was the only method available and, for reasons to be explained presently, it was limited to amplitudes in excess of 1 µm (Essentially the same limitations applied to Wilska s 1935 experiments) Thus, Bekesy s experiments were carried out at SPLs of 130 dB and even higher, i e, at 'non physiological' levels The threshold shown in Fig 1 represents an extrapolation over seven orders of magnitude, i e, over a range of 1 1/10 millionth Both the SPLs employed and the extrapolations required raised considerable scepticism about the validity of these earlier results

It became necessary therefore to develop new methods with much higher sensitivities It may already be mentioned here that, after such methods become available, it was possible to take measurements on the tympanic membrane at amplitudes approaching the threshold values of Wilska s (Fig 1) For example, results obtained in cats at levels of 10 Å to 0 1 Å with the aid of laser interferometry indicated that the earlier measurements had not been in any gross error by giving values that were too small (Tonndorf & Khanna, 1968)

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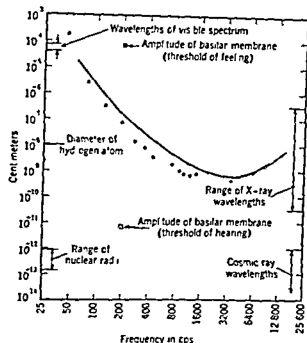


Fig 1 Displacement of the tympanic membrane at the auditory threshold over a range of frequencies (data from Wilska, 1935) and those of the basilar membrane at 200 Hz, both at the threshold of feeling and that of perception (data from Békesy, 1947) Some magnitude of atomic and subatomic structures and/or events have also been entered (from Bekésy & Rosenblith, 1951)

Before these new methods can be described in some detail, it might be necessary to make ome general remarks about the difficulties involved in such low-level measurements

LIMITATIONS OF SMALL AMPLITUDE MEASUREMENTS

Mechanical responses as a rule do not have "thresholds" in the accepted sense They simply grow smaller in direct proportion to the decreasing input, the relation being a perfectly linear one However, there are *pseudo-thresholds*, i e, *limits of detection* their levels vary with the method used.

For example, optical instruments have limited *resolving powers* Resolving power is defined as the minimal separation between two objects for which they appear distinct and separate when viewed through the instrument in question The ultimate limit in this case is given by the wavelengths of visible light, 4 000 to 8 000 Å (It was the latter that constituted the limits of Wilska's and Békesy's measurements) Due to the much shorter wavelengths of their electron beams electronmicroscopes have much improved resolving powers, in the range of 1 Å and less

More generally speaking, the limit is given by the *background noise* Its sources are manifold and may include the following

(*a*) *mechanical vibrations*, a quasi broad band noise with emphasis in the low frequencies, (*b*) the so called "*resistance noise*" of associated electronic circuits, a true broad-band noise, and (*c*) the *power-line hum*, a 60 Hz harmonic spectrum event While there are ways of keeping all three of them at minimal levels by (i) sturdy and *shock-proof mounting*, (*ii*) choice of low-noise electronic components and shielding, both electrostatically and electromagnetically, and (iii) appropriate circuit design, shielding and careful grounding of the electronic equipment, there is always a residual value Further reduction is then possible by means of *narrow-band filtering* (sine-wave responses) or *signal averaging* (broad-band responses) Speaking electrically, the current state of-the art permits reduction of the noise levels in narrow bands (less than one cycle wide) to the upper nanovolt range

The worst "noise" (because it has all the properties of the expected response and is therefore not easily detected) is signal leakage from the input system into the response system It may be brought about by vibrations that are picked up mechanically or by direct electrical leakage While leakage will never be zero, one must check one's system carefully to make sure that there is none at levels within at least −20 dB of the lowest responses one wants to register Improvements if indicated are not always easy to achieve

If resolving power applies to the *spatial* parameters of the event under study, *frequency limitation* may be considered its equivalent in the *time* domain The detecting system must have a frequency range compatible with that of the signal to be employed, preferably a wider one The

human eye with its flicker-fusion rate of about 15 impulses/sec is severely limited in this respect If one deals with sine-wave events, one may to good advantage employ stroboscopic illumination in order to compensate for this shortcoming Another method one can use is called *fuzziness detection*, the registration of just noticeable displacements at the visual threshold of the observer Under magnification, one focuses on some small details of the structure under observation and registers the magnitude of the input signal at the level at which these targets become fuzzy while they are vibrating Using ordinary light, Tonndorf (1957) obtained a sensitivity of about 1 μm Using *laser* illumination and observing the fuzziness of the resulting *speckle* pattern, Kohlloffel (1972) reached a sensitivity of about 0 11 μm (1 1 × 10^{-5} cm) for peak-to-peak displacements, a distinct improvement (*speckle interferometry*)

We are now in a position to discuss modern techniques This discussion will include the following Measurements at one single point (capacitive probe, Mössbauer effect method, laser interferometry and heterodyne spectroscopy) and assessment of vibratory patterns (time-averaged and real time holography) Finally the advantages and disadvantages of these methods will be discussed

MEASUREMENTS AT ONE SINGLE POINT

Capacitive Probe

The first method with an improved sensitivity was the capacitive probe of Backhaus (1930) It was first adapted by Bekesy (1941) for the measurement of tympanic-membrane displacements It has since been employed (and technically improved) by other investigators in similar studies (Fishler et al, 1967) and also in measurements of basilar-membrane displacements (Wilson & Johnstone, 1975)

Fig 2 shows the principle of operation The object of interest forms one plate of capacitor *C*, a small plate brought close to it forming the other (distance between plates 0 5 mm Békésy, 1941, within 20 μm Wilson, 1973) This capacitor is part of a circuit in which a high frequency current is flowing (100 kHz Békésy; 1 mHz Wilson) When the object is vibrating with respect to the stationary small plate, the value of the capacitor alters in direct proportion to the change in distance between the two plates This change produces a frequency modulation of the carrier signal, the modulating signal being the displacement waveform The latter is detected in exactly the same manner as in the detecting stage of a commercial FM radio set, i e, by conversion into an amplitude modulated signal

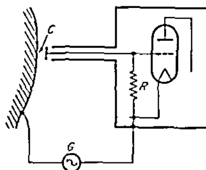


Fig 2 Schematic representation of the capacitive probe, for details cf text (from Bekesy, 1941)

The probe must be calibrated in a separate set up against a plate vibrating with a known displacement In Bekesy's hands, the sensitivity of the method was about 10^{-6} cm (=100 Å) Fishler et al (1967) improved this to 2 Å There is a direct read-out and errors of observation are reasonably small, i e., ±1 dB or less

As well as being displacement sensitive, the capacitive probe is capable of registering phase angles with respect to a reference signal

The Mössbauer Effect

A method employing the so-called *Mössbauer effect*, as first advocated by Hillman et al (1964), represented a second major improvement Gilad et al (1967) first used it for the measurement of tympanic membrane displacements Johnstone & Boyle (1967), Johnstone et al (1970), Rhode (1971), and Helfenstein (1974) applied the same method to measurements of basilar-membrane displacements

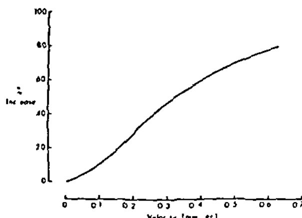


Fig 3 Velocity of a vibrating object vs percent increase in frequency counts (from Johnstone et al, 1970)

A small radioactive source is pegged onto the object in question A nearby absorber and counting system register the gamma-rays it emits With the object vibrating, the changing *velocity* of the source creates a *Doppler* effect, i e, the gamma ray count is increasing as the source approaches the absorber and vice versa

The sources employed by Gilad et al (1967), were thin pieces of ^{57}Co diffused in copper, 0 3 mm in diameter, and weighing about 0 1 μg For intracochlear usage, the size had to be reduced Helfenstein, for example, employed sources of 30 × 30 × 3 μm, weighing approximately 0 5 μg ne gamma rays emitted by such sources are werful enough to penetrate approximately 1 cm of soft tissues or 1 mm of bone Although the output is proportional to the velocity (v) of the object, its displacement (d) can be easily calculated, since $d = v/2\pi f$ (f frequency)

Fig 3 gives a typical calibration curve, i e, source velocity vs percent increase in counting. Note the non-linearity of the curve The method has a limited linear range, in Fig 3, for example, less than 20 dB In the hands of Gilad et al outputs were linear over a range of 30 dB Rhode reported (pers comm, 1975) that he is now able to achieve a linear range of about 35 dB [For the sake of comparison, the dynamic of visual measurements is rarely better than 26 dB (=1 20), Tonndorf, 1957]

By definition, the "responses" consist of series of discrete pulses randomly distributed in time and fluctuating in magnitude, i e, the read out is very noisy, obscuring the actual waveform Therefore, one has to go through a lengthy averaging process, usually over periods of one to two cycles, for total durations of 5 to 10 min Then, the reading accuracy is approximately 10 %, i e, ±1 dB The averaging process allows also to obtain phase data Typical optimal values (SPL at the input, frequency, and displacement), as obtained by Johnstone et al, are given in Table I Note the improvement in sensitivity with higher frequencies In a velocity-sensitive system, displacement increases with frequency at 6 dB/octave

Table I *Some typical sensitivity data of basilar membrane displacement (from Johnstone et al, 1970)*

85 dB	6 kHz	150 Å
55 dB	20 kHz	10 Å

Optical Methods

Laser interferometry

The advent of the laser with its coherent light (i e, light that is *in* phase over the entire wave front of its beam) made it possible to improve, by many orders of magnitude, the sensitivity of optical interferometry, a time tested old method (Michelson, 1890) In the ear, laser interferometry was first applied to measurements of tympanic membrane displacements, i e, those of the umbo (Khanna et al, 1968, Tonndorf & Khanna, 1968)

The principle is shown in Fig 4 After de-

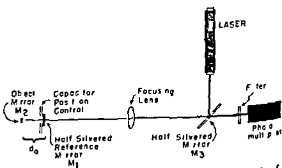


Fig 4 Principle of laser interferometry for details cf. text (from Khanna et al, 1968)

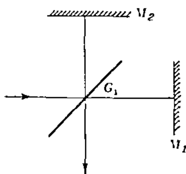


Fig 5 Michelson's original interferometric method The two mirrors were physically separated, requiring ex tremely sturdy mountings, for further details cf text (from Francon, 1966)

flection by 90° at half-silvered mirror M_3 and focusing, part of the laser beam is reflected from half silvered mirror M_1, the reference mirror, the remainder from object mirror M_2 By appropriate and very accurate leveling of both mirrors, the two reflected beams are made to coincide precisely on the face of the photomultiplier If now mirror M_2 vibrates with respect to (stationary) mirror M_1, a time-varying interference is set up between the two beams Its waveform is registered by the photomultiplier tube If the average distance, d_0, between M_1 and M_2 is adjusted to an exact multiple of $\lambda/4$ of the laser light ($\lambda = 6\,328$ Å for helium-neon lasers), the output waveform of the photomultiplier is maximized and is then an exact replica of the vibratory waveform

In the original Michelson method (Fig 5), mirror M_1 had been placed straight in the original beam, beyond M_3 and far away from M_2 For the present purpose, this method proved to be far too noisy, since animal skulls are not rigid enough, and the two reflecting mirrors, M_1 and M_2, then move independently of each other in a random manner Fastening M_1 directly over object mirror M_2 to the same frame of reference, i e, to the animal's skull (Fig 4), minimized independent movements of the two mirrors, thus reducing the noise

Since there have been some misunderstandings about the degree of sensitivity that can be achieved by laser interferometry (Dallos, 1974, Fex, 1974, Stark, 1976), some elaboration of this point may be in order

The interferometer output as a function of the vibration amplitudes of M_2 is given by the *sine* of a *sine* function, a so called *Bessel* function The present method employs a first-order Bessel function of the first kind, a function that forms a characteristic series of maxima and minima (Fig 6) For a helium-neon laser, the first maximum occurs invariably and exactly at an amplitude of $0\,92 \times 10^{-5}$ cm or about 1 000 Å The first minimum at $1\,92 \times 10^{-5}$ cm is very sharp (as are all subsequent minima) and may

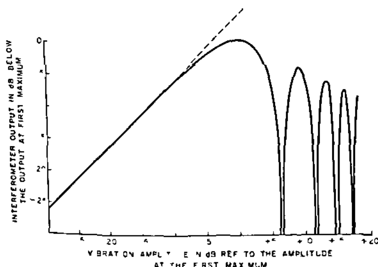


Fig 6 Interferometer (photomultiplier) output as a function of the vibration amplitude, both are given in relative terms in reference to the voltage or amplitude at the first maximum (from Khanna et al, 1968)

thus be utilized as a convenient point of *built-in* calibration Below the first maximum, i e, from about 20 dB off the top on downward, the slope is perfectly linear, and the non-linearity at the top end of the curve is known precisely. For this reason, any amplitude that is *smaller* than that of the first maximum can be read with great precision as a *point in a continuum* The strength of the technique is in taking readings at amplitudes *very much* lower than 10^{-5} cm There is no lower limit, except noise

The present improved instrument (Khanna, 1976, unpublished data) permits stable readings *without any filtering* at RMS displacements amplitudes of approximately 10^{-8} cm (1 Å) and a S/N ratio of about 15 dB By means of appropriate narrow-band filtering, this value can be extended downward to 10^{-11} cm (10^{-3} Å) and, if so desired, beyond that value As already stated, there is no real limit Signal averaging, a time-consuming process, is not really needed with this method, although it might improve sensitivity further Therefore, readings can be taken at a relatively fast rate Since a waveform is being recovered, phases can also be measured

The experiments on the tympanic membrane (Tonndorf & Khanna, 1968) permitted the use of relatively large mirrors thin slices of silvered mica 0 4 mm in diameter, and weighing slightly more than 1 μg For experiments on the basilar membrane now in progress in this laboratory, gold crystals, 20 to 40 μm in diameter, were chosen They are optically flat, but extremely thin (thickness in the range of 10 nm) Hence their weights are also extremely small, i e, in the fractional nanogram range

Optical heterodyne spectroscopy

A related, but somewhat different technique, so-called optical heterodyne spectroscopy, has been employed by Dragsten et al (1974) on the auditory organ of some cricket This method, instead of utilizing the direct reflections from two separate mirrors, allows the vibrating structure to scatter the laser light No mirror needs to be attached to that structure The detection method is more complex than that of the previous method, both optically and electronically Sensitivity was, according to the authors reported to be around 1 Å with extremely narrow filtering, necessitated by the inherent noisiness of the method The dynamic range was 70 dB and the spatial resolution 10 μm

Assessment of Vibratory Patterns

All methods discussed so far permit measurements of displacement amplitudes at *one point* Sometimes it is desirable to assess *whole displacement patterns* over large areas for given frequencies and input amplitudes

The classical visual observations permitted to do that to some extent The eye can quickly scan over the entire visual field and determine, for example, the spatial spread of a given vibratory event Phase comparisons can also be made (Bekesy, 1947) *Speckle interferometry* as utilized by Kohllöffel (1972) was already mentioned It can be employed in essentially the same manner with an improvement in sensitivity of approximately one order of magnitude (cf above) Phase read-outs can also be obtained with this technique

Time-averaged holography

This method, first introduced by Powell & Stetson (1965) for the assessment of vibratory patterns, is one of great precision The method was first applied to measurements on the tympanic membrane by Khanna (1971), Khanna & Tonndorf (1972), Tonndorf & Khanna (1972) It has also been used on insect ears by Michelsen (1971)

Fig 7 shows the instrumental arrangement as used by Khanna & Tonndorf A laser beam is split into two parts, each of them is then made to diverge The *reference beam* falls onto a photographic plate of spectrographic quality The *object beam* falls first onto a *diffusely reflecting* object whence it goes likewise to the plate Here an interference is set up at every point of the plate, each of them carrying the *entire* information in a coded form, hence the

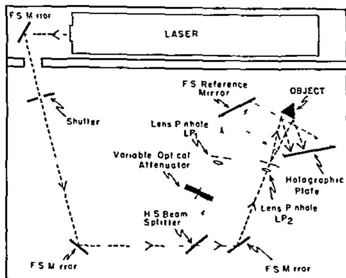


Fig 7 Experimental arrangement for time-averaged holography on small objects (from Khanna & Tonndorf, 1971)

term "*hologram*". When the object is being vibrated during photographic exposure (for example, there will be 100 periods of a 1 000 Hz signal during 1/10 of a sec exposure) the average peak to-peak displacement is being recorded as seen by any given point of the plate After development of the holographic plate, an optical diffraction grating is obtained If one looks through this grating illuminated by a divergent laser beam, the image is reconstructed, and superimposed upon it is a bright/dark fringe pattern. The latter can be seen and/or photographed in this manner An example is given in Fig 8 Each of the alternate light and dark interference

600 Hz 111 dB



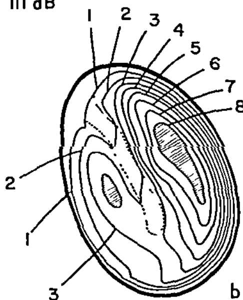


Fig 8 (*a*) Time averaged hologram of a cat s left tympanic membrane, slightly retouched for better reproduction. (*b*) Schematic drawing The malleus runs down from the top left In both instances, the numbers refer to the sequence of dark fringes (from Khanna & Tonndorf, 1971)



Fig 9 Real time (*left*) and time averaged holograms on an earphone diaphragm for a given set of frequencies Beyond the resonant frequency (765 Hz in the present case) the patterns became highly complex (from Khanna et al 1973)

fringes represent an *equal amplitude contour* so that the entire displacement pattern can be assessed in every detail

Admittedly, the sensitivity of the method is not as large as that of the modern one point method, i e, only $1\,92 \times 10^{-5}$ cm for helium-neon lasers, but recording whole displacement patterns at once has other obvious advantages

Phase readings can be obtained by taking a series of holograms under stroboscopic illumination and at varying phase angles Since the latter is a highly time-consuming method, such readings have not yet been obtained in the ear It is known from the time of Lord Rayleigh (1877) that each nodal line, as shown for example in the patterns of Fig 9, separates vibrating areas that are 180° out of phase with each other so that detailed phase information is not really that important

Real time holography

Real time holography (i e, in which the pattern is observed immediately and directly) has also been developed, e g, Khanna et al (1973) So far they have not been employed in studies on the ear For the benefit of the real time method it is first necessary to obtain a stationary hologram of the object and then to view the object through it At rest, both image and object must precisely coincide This precise alignment has not been achieved with the tympanic membrane because of its low impedance and its tendency to slow quasi d c drifts When the object is being vibrated a set of displacement fringes become visible Fig 9 shows a comparison of patterns obtained on an earphone diaphragm by the real time method and the time averaged technique

DISCUSSION

When compared with the classical method of visual measurement all the newer one point methods described have the advantage of much improved sensitivity Some of them are equivalent in sensitivity to electronmicroscopic resolution, or even exceed it All of them permit phase readings to be obtained Their frequency ranges are ample and, with the exception of the Mossbauer method so are their dynamic ranges They do not seem to load the structure under study Even with the methods that employ

gamma ray sources or mirrors, the weights of these attachments seem hardly large enough to cause any appreciable loading, although this point has not been satisfactorily checked for all of the methods, neither has the question been answered as to how well these attachments were affixed to the vibrating structure Helfenstein (1974) used a biological glue assuring good fixation to the basilar membrane, but his phase data give rise to the suspicion that he may have adulterated the membrane One disadvantage common to all these methods is that they are invasive, i e , the cochlea has to be opened Al though the output of the Mössbauer source could probably be read through the osseous cochlear capsule of small animals like the guinea pig, the capsule had still to be opened for the placement of the source With an organ that is relatively inaccessible this disadvantage can hardly be avoided Once more, however, no tests have been carried out to check what ef fects, if any, the opening of the cochlea might have on the sensitivity and/or the displacement pattern of the basilar membrane

One heavy price the experimenter has to pay for the high sensitivity and the great precision of these modern methods is that he loses all "feel" for the results obtained With the classical visual methods, the experimenter knew im-mediately when the structure under study was vibrating, and he was usually able to recognize the mode of such vibrations, because he observed them directly Today one can no longer be so sure The possibility of falling victim to experi-mental artifacts has been commented on above Without exception, the electrical outputs of the devices under consideration, for the SPLs ap-plied, are very small, making the issue of S/N ratios and their improvement an important one One must be thoroughly familiar with the method *and* its theoretical foundations to optimize its use and to minimize the chances of experimental artifact Ancillary experiments employing com pletely independent approaches are needed to check the reliability of the method and to define its limitation It is highly desirable that the same phenomenon be investigated by means of dif-ferent methods in an effort to find out if the results are compatible with one another

The last remark already points to an answer to the question, i e , which of these methods might be the best from an all around stand-point Each of them has its individual advantages and disadvantages (cf above) However, we in this laboratory are most impressed by (but of course are also most familiar with) laser inter-ferometry In our opinion (Khanna & Tonn-dorf), it is more accurate than the other methods on account of its built-in calibration and more reliable on account of its wide dynamic range, but it is also more demanding with respect to its instrumental requirements (alignment)

Some of the methods presented have not yet been applied to the ear They were nevertheless included because of their *potential* value for auditory research

Finally, the author may be permitted a per-sonal observation The great complexity of these methods make it rather doubtful that, given no prior knowledge about mechanical cochlear function, they would have led directly to the establishment of the traveling wave concept, without that a number of ancillary experiments, including old fashioned, direct microscopic ob-servations, would have been carried out

RÉSUME

Les deplacements de la membrane basilaire, provoques par le son au niveau du seuil auditif, comportent des fractions d Ångstrom L'enregistrement visuel, utilise par Bekésy dans ses recherches fondamentales, est limite aux valeurs en dessus de 10 000 Å Actuellement nous dis-posons de deux methodes ultra microscopiques 1 l ap-plication de l'effet de Mossbauer, qui a été utilise avec succès par plusieurs auteurs, 2 l interférométrie au Laser qui est actuellement adaptee au mesurage au niveau de la membrane basilaire, elle a dejà été utilisee pour des mesurages au niveau du tympan Les avantages et les desavantages des deux methodes sont mis en discussion

ZUSAMMENFASSUNG

Auslenkungen der Basilarmembran verursacht durch Schallintensitaten an der Horschwelle, betragen Bruch-teile eines Ångströms Visuelle Registriermethoden wie sie von Bekésy in seinen grundlegenden Untersuchungen benutzt wurden, sind definitionsgemäß auf Werte ober-

halb von 10 000 Å begrenzt Die vorliegende Arbeit beschreibt eine Zahl von modernen Methoden, die in der Lage sind, Werte im niederen Ångström Bereich zu messen Ein-Punkt-Methoden (kapazitive Sonde, Mössbauer-Methode, Laser Interferometrie und optische heterodyne Spektroskopie) sowie Methoden zur Erfassung von Auslenkungsmustern („time averaged" and „real-time"-Holographie) Vor- und Nachteile dieser Methoden werden diskutiert

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DISCUSSION

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THE PATAS MONKEY AS A MODEL FOR DIHYDROSTREPTOMYCIN OTOTOXICITY

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Abstract Although the cochlear toxicity of dihydrostreptomycin (DHSM) is well recognized in man, it has always proved difficult to demonstrate in animals Hearing thresholds in *M nemestrina* monkeys remained essentially unchanged after DHSM 100 mg/kg im daily for 8 months, but *E patas* monkeys were severely deafened by DHSM 20 mg/kg for 90 days, a regimen formerly used in treating human tuberculosis The patas monkey may prove to be the animal model of choice for evaluating aminoglycoside ototoxicity

Dihydrostreptomycin (DHSM), introduced with so much optimism in 1948 as an equally effective but less vestibulotoxic version of its parent aminoglycoside streptomycin (SM) (Edison et al, 1948, Hobson et al, 1948, Hinshaw et al, 1948) became in the course of a few years a virtual pariah among antibiotics because of its insidious and sometimes delayed cochlear toxicity (Allison et al, 1949, Shane & Laurie, 1950, Glorig, 1951, Liden, 1953) Double-blind studies by Cohen et al (1953) and Mahady et al (1953) left no doubt as to the greater incidence of high-frequency hearing loss in tuberculous patients after prolonged treatment with DHSM, in contrast to the prevalence of vestibular disturbance in those receiving SM Ultimately, the use of DHSM was condemned, after Shambaugh et al (1959) collected 37 cases of sensorineural hearing loss in patients who had received only a few injections in combination with penicillin for relatively minor infections

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Despite its frequency of occurrence in man, the cochlear toxicity of DHSM has been more difficult to demonstrate in the cat and guinea pig, species commonly used for ototoxicity tests in the laboratory, than that of kanamycin or neomycin, or even SM itself (Hawkins & Lurie, 1952, 1953, McGee & Olszewski, 1962, Tyberghein, 1962) The reason for this curious discrepancy is by no means clear, especially since DHSM has been shown to achieve a relatively high concentration in the perilymph of the guinea pig after parenteral injection (Stupp, 1970)

The development of hearing loss during aminoglycoside treatment can be followed in monkeys of the genus *Macaca*, which are trained for behavioral audiometry by positive reinforcement techniques (Stebbins et al, 1969, 1973) The present experiment was prompted by the incidental finding of a severe hearing loss in an *Erythrocebus patas*, i e non macaque monkey given DHSM The paired macaque (*M fascicularis*) given identical treatment showed little or no change in hearing thresholds For the first time ototoxic effects are demonstrated in a laboratory species given an aminoglycoside in doses as small as those recommended for clinical use

SUBJECTS AND TEST PROCEDURES

Seven *E patas* ("military") monkeys (3 0–5 3 kg), four *M nemestrina* ("pigtail") (2 5–5 1 kg), and one *M fascicularis* ("crab-eating") (M-27, 3 4 kg) were used in this experiment They were

trained by methods previously described, and all had normal pretreatment audiograms As in the study of noise-induced hearing loss recently reported (Hawkins et al, 1976, Moody et al, 1976) thresholds were measured with the animal seated in a primate restraining chair inside a double-walled sound chamber (Industrial Acoustics Company) Calibrated earphones carefully fitted over the openings of the ear canals permitted each ear to be tested separately

As in previous work with monkeys the positive reinforcement used consisted of banana-flavored food pellets (Noyes) Experimental contingencies were controlled by a small computer (PDP-8/L) Pure-tone stimuli were generated by a bank of nine oscillators (HP 204C), attenuated by a programmable attenuator, and gated by a tone switch having a rise-fall time of 50 msec The ear phones were calibrated by a probe-tube microphone inserted through the cushion so that the opening of the tube was located directly in front of the animal's ear canal

Threshold tests included both tone-trials and catch-trials Responses made to catch-trials when the tone was off gained no reward and were further punished by a 5 sec delay of the ext trial As in the past, a form of Bekesy udiometry involving a tracking procedure was sed Thresholds for nine frequencies were measured in each daily session, which lasted about one hour

Drug administration

The DHSM sulfate used was obtained as a gift from the Upjohn Company and later obtained by purchase from Nutritional Biochemicals, Inc It had a stated potency of 800 μg DHSM-base per mg Dissolved in water for injection USP, it was given once daily by the intramuscular route Injection sites were rotated to minimize local irritation

Treatments received by the individual animal subjects are shown below in Tables I and II The doses of 40 and 20 mg/kg/day correspond approximately to 2 and 1 g/day, respectively, for human patients The latter dose level had been widely used in the treatment of tuberculosis, the courses often lasting for 120 days or more, as in the double-blind studies cited above In the macaques drug treatment was continued for 120 to 240 days, in the patas for 120 days or less, depending on the severity of the ototoxic or nephrotoxic reaction

Cochlear histological examination

Threshold measurements were continued after the end of DHSM treatment until it was clear that no further change was occurring The column "post Rx days" in Tables I and II shows how long each animal was followed before being anesthetized with pentobarbital sodium for euthanasia The membranous labyrinth was fixed and stained *in vivo* by perilymphatic perfusion of 1% OsO_4 solution (Zetterqvist) Cochlear tissues were prepared by microdissection as described by Hawkins & Johnsson (1976) and whole mounts (surface preparations) of Corti's organ were examined by phase contrast microscopy A complete count of the hair cells and phalangeal scars was made, and the percentage of hair cells remaining in each row per mm of length was plotted as a cytocochleogram

RESULTS

Macaques

As seen in Table I, only minor changes occurred in the audiograms of the 2 animals receiving 100 mg/kg/day, even though this dose was given for 240 days and threshold measurements were made for 96 and 128 days respectively, after DHSM was discontinued

In the two ears of M-70 there were threshold shifts of 40 or 45 dB at the highest frequency (40 kHz) but less than 10% of the hair cells were absent at the basal end of the basilar membrane There was also a broad, shallow loss of 10 to 20 dB at frequencies from 60 to 2 000 Hz but no corresponding hair cell loss was seen in the upper turns (Fig 1) The ears of M 40 showed hearing loss of 10 and 15 dB at 60 Hz, which was accompanied by a sharp loss of inner hair cells restricted to the apical portion of the cochlea (Fig 2)

Table I *Macaca sp*

No	Days	Post Rx days	Ataxia	Extent of HL (Hz)	Extent of HC loss ihc	ohc
100 mg/kg/day						
M-40	240	96	no	low freq, 40 k	apex	sl
M 70	240	138	no	40 k	sl	sl
40 mg/kg/day						
M 27	194	192	no	no	—	
M 35	200	171	no	no	—	
20 mg/kg/day						
M 93	120	52	no	no	—	

In the 3 monkeys of this genus that received DHSM 20 and 40 mg/kg/day, hearing thresholds remained unaffected Only one of these animals has been sacrificed, and the cochlear examination has not been completed In view of the findings in M-40 and M 70, it is unlikely that significant hair cell changes will be found

None of the macaques showed evidence of vestibular dysfunction at any time during or after DHSM treatment

Patas

In all 7 of the patas monkeys the effect of DHSM on hearing was severe, as seen in Table II The first shifts in high frequency thresholds which could be regarded as significant, i e 20 dB or more at 32 kHz, appeared after 7 to 10 weeks of treatment Hearing loss was progressive both during and after treatment Eventually all frequencies were affected in 3 of the animals In the other 4, hearing for at least some of the lower frequencies was spared

Widespread loss of hair cells was found post mortem In the 3 animals which showed hearing loss at all frequencies (M-44, M 62, and M 91) inner hair cells were almost entirely absent, and outer hair cells remained only at the apex In the other 4, which had retained hearing for low frequencies, there was a striking disparity of effect on inner and outer hair cells As seen in Fig 3, the abrupt hearing loss for frequencies above 1 kHz shown by M-45 was

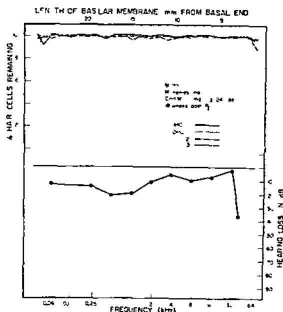


Fig 1

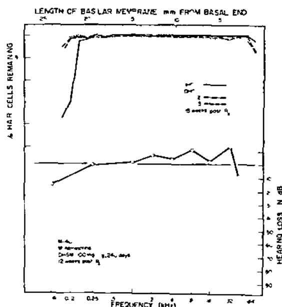


Fig 2

Figs 1 2 Final audiograms (*below*) and cytocochleograms (*above*) for macaque monkeys treated with DHSM [illegible]

associated with a complete loss of outer hair cells below 16 mm, whereas inner hair cells disappeared only below the 5 mm point Thus

Table II *Erythrocebus patas*

No	Days	Post Rx days	Ataxia	Extent of HL (Hz)	Extent of HC loss (mm from base) ihc	ohc
100 mg/kg/day						
M-44	100	24	yes	all	~all	20
M 61	91	138	no	>500	16	18
M 62	75	88	yes	all	26	25
40 mg/kg/day						
M-45	120	92	no	>1 000	5	16
M 81	120	92	yes	>250	15	20
20 mg/kg/day						
M 91	102	187	no	all	~all	~all
M 92	90	123	no	>250	5	20

throughout most of the basal turn only inner hair cells were present An even greater difference in the extent of loss of inner and outer hair cells was seen in M-92, which retained normal threshold only at and below 250 Hz, with a sloping audiometric curve above that frequency (Fig 4)

All of the animals retained some hearing at the time the DHSM injections were stopped, but M-44 responded only at frequencies of 0 5 to 8 kHz at hearing levels of 50 to 65 dB In the others there was a definite further progression of the hearing loss, as seen in Figs 3 and 4) The most dramatic post-DHSM loss occurred in both ears of M-62 By the time DHSM was discontinued this animal had developed a sharply defined threshold shift of 30 to 50 dB for frequencies above 4 kHz Below 2 kHz there was a broad, shallow loss of 10 to 25 dB Unfortunately, a vestibular disturbance (see below) caused M-62 to stop working, so that no hearing thresholds could be recorded for nine weeks When audiometry was resumed he showed a loss of 60 to 70 dB even at 250 Hz, and no response at higher frequencies Examination of the cochlea revealed only a few hair cells remaining at the apex (Fig 5)

Vestibular and renal disturbances

Obvious disturbance of vestibular function was observed in three of the seven patas monkeys

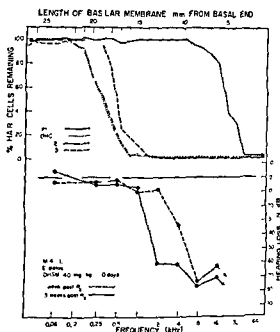


Fig 3

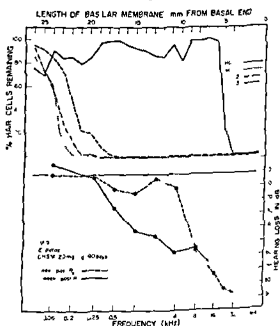


Fig 4

... for E. ... corded at one week had spread by 16 weeks to include all frequencies above 250 Hz, with complete loss above 8 kHz (In all of the figures placement of the logarithmic scale for frequency with respect to the linear scale for length of the basilar membrane is arbitrary, but it gives a reasonable approximation of the correspondence between hearing loss and hair cell loss)

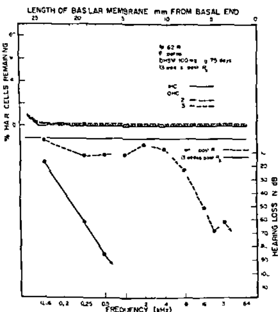


Fig 5 Audiogram and cytocochleogram for a patas monkey treated with DHSM in the same dose as the nemestrinas M 70 and M-40 in Figs 1 and 2 Between the 1st and 13th weeks after DHSM hearing was completely lost for all but the lowest frequencies The cytocochleogram shows only a few outer hair cells remaining at the apex

(Table II) In one it appeared during DHSM treatment, in the other two afterwards M-44 began to perform his audiometric task poorly on the 71st day of the injections and showed a definite loss of balance by the 77th day His BUN was normal at that time, but epithelial casts and albumin appeared in the urine by the 95th day Later urine samples were normal, and the BUN was 17 5 mg% M-81 was ataxic and had difficulty climbing into his chair 16 days after the course of DHSM Fortunately, he was willing to continue the audiometry on a regular schedule for 3 months without interruption

One week before his last injection of DHSM, M 62 was working erratically and spitting out the food pellets he received as reinforcements Five days after the last injection he had a BUN of 46 mg%, 2 days later he was staggering and apparently dizzy Because he continued to eat poorly and to lose weight, he was given daily injections of dimenhydrinate (Dramamine[3]) 5–10 mg i m , and occasional injections of a preparation of vitamin B complex After 2 weeks he began eating again and after 4 weeks the injections could be continued and threshold measurements resumed

Stria vascularis and spiral ligament

In the patas monkeys the suprastrial portion of the spiral ligament showed microvascular changes, i e intervascular strands and avascular channels replacing suprastrial capillaries, such as we have described in guinea pigs treated with aminoglycosides (Hawkins, 1973) There was also evidence of loss of superficial cells from the ligament in this area In both patas and macaques the stria vascularis, especially in the basal turn, showed patchy atrophic changes The stria had also receded from the attachment of Reissner's membrane, leaving a margin of atrophic epithelium These changes, which were not seen in an untreated patas used as a control, do not necessarily represent the severity of strial injury at the time DHSM treatment was concluded, since the stria has considerable powers of recovery from injury (Duvall et al, 1974) Inasmuch as strial changes were observed in both groups of DHSM treated animals, the question of a causal relation between strial injury and hair cell degeneration remains unanswered

DISCUSSION

The abrupt hearing losses which were present in the patas monkeys M 62 and M 92 at the end of DHSM treatment, and which persisted in M-45, are reminiscent of our own earlier group of macaques treated with kanamycin (KM) and neomycin (NM) (Stebbins et al, 1969) Nevertheless, there are interesting differences between the stability of the hearing losses recorded after KM treatment and the progressive deterioration of hearing after DHSM, although a similarly progressive and eventually almost complete hearing loss occurred in the NM treated monkey M-21 in the earlier study There is also a difference in the patterns of hair cell loss caused by KM and DHSM

With KM the inner and outer hair cells were affected almost equally, but with DHSM the loss of inner hair cells was much less, at least in M-45, M-61, M-81 and M-92 The pattern of loss in M-45 (Fig 3) resembles that in chinchillas and guinea pigs after KM (Ryan & Dallos, 1975, Hawkins et al, 1976) At the extreme apex, however, the outer hair cells appear to be hardier than the inner, whether the damage be minor, as in the macaque M-40, or catastrophic as in the patas M-44 and M-62 In all of the animals both hearing losses and hair cell losses showed a striking symmetry of pattern for the right and left ears, just as in the previous study

The abrupt hearing loss for frequencies above 1 kHz in M-45 and the sloping audiogram recorded 16 weeks after DHSM in M-92 (Fig 4) offer an instructive comparison Except for the most basal 5 mm, the inner hair cell population is almost intact in both animals In M-45 the outer hair cell rows are virtually complete in the last 5 mm of the upper turns, whereas in M-92 their loss is more extensive, and outer hair cells are missing from all three rows even at the extreme apex The findings are compatible with the generally-accepted hypothesis that normal thresholds of hearing represent the responses of the outer hair cells, whereas the inner hair cells respond only at much higher intensities (Ryan & Dallos, 1975)

To account for the sloping curve in M-92 one may speculate that in the absence of the outer hair cells the inner hair cells of the middle turn have somewhat lower thresholds for 0 5 and 1 kHz than those of the basal turn for 4 and 8 kHz It is not clear, however, why the threshold for 2 kHz should be 15 dB higher in M-45 than in M-92, unless one assumes that subtle changes had occurred in the inner hair cells as a result of the 40 mg/kg dose of DHSM given M-45, which were not revealed by the light microscope We have seen such changes in the sterocilia of inner hair cells that remained after KM treatment in guinea pigs (Hawkins, 1976)

Hair cell patterns like those found in M-45 and M-92, with a long row of inner hair cells persisting in the absence of outer hair cells, should be ideal for testing current hypotheses concerning the recruitment of loudness Unfortunately the animals in the series had not been trained for reaction time measurements, but we plan to make a study of recruitment by Moody's (1973) method in other DHSM treated patas monkeys with audiograms like those of M-45 and M-92 The reason for the greater sensitivity of the patas monkey to DHSM remains something of a mystery We had supposed that the patas, a native of arid sub-Saharan Africa must have kidneys adapted to the most frugal use of water, and may therefore excrete the antibiotic less rapidly than the nemestrina, which is accustomed to the water abundance of the Indonesian rain forests The hypothesis seemed attractive, especially since water consumption tests show a higher turnover for the nemestrina than for the patas DHSM blood level measurements on the other hand, at various stages of prolonged administration, have yielded essentially the same results for both species The concentration curves fell at the same rate for both and neither showed any detectable DHSM in the plasma after 24 hours The possibility remains that perilymph levels of DHSM may be higher or more enduring in the patas, but such measurements are still to be completed

What has been clearly established is that the patas monkey presents an excellent and thus far unique model for DHSM ototoxicity Indeed, the patas is the first species with a cochlear sensitivity to an aminoglycoside comparable with, and in fact greater than, that of most patients Whether it will show a similar sensitivity to other aminoglycosides and thus reveal itself as a species of choice for ototoxicity testing prior to clinical trial remains to be determined

RESUMÉ

La toxicité cochleaire de la dihydrostreptomycine (DHSM) chez l homme est bien connue mais sa démonstration chez des animaux a été toujours difficile Les seuils auditifs des singes *M nemestrina* sont restés essentiellement normales apres traitement de DHSM à 100 mg/kg par voie intramusculaire pendant 8 mois En revanche des singes *E patas* ont montré une surdité profonde apres traitement de DHSM à 20 mg/kg/jour pen-

dant 90 jours, régime utilisé, il y a quelques années, dans la thérapie de la tuberculose. Le patas est peut-être l'animal qui permet le mieux d'évaluer l'ototoxicité aminoglycosidique.

ZUSAMMENFASSUNG

Die Toxizität des Dihydrostreptomycins (DHSM) für die menschliche Schnecke ist allgemein bekannt, doch ist es immer schwierig gewesen, diese Ototoxizität in den üblichen Versuchstieren zu demonstrieren. Bei *M. nemestrina* Affen blieben die Hörschwellenwerte im wesentlichen ungeändert nach parenteralen Gaben von 100 mg DHSM pro kg täglich über 8 Monate. *E. patas*-Affen dagegen zeigten schweren Hörverlust nach Behandlung mit 20 mg DHSM pro kg über 90 Tage, eine früher übliche Dosierung bei der Tuberkulosetherapie. Der Patas-Affe ist möglicherweise das Versuchstier der Wahl für die Bewertung der Ototoxizität der Aminoglycosid Antibiotika.

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DISCUSSION

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ELECTROCOCHLEOGRAPHY AND COCHLEAR PATHOLOGY

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Abstract In experimentally damaged inner ears the structural alterations were correlated to electrocochleographic responses of the ear Sectioning of the cochlear nerve with degeneration of the type I neurons but intact sensory cells results in normal cochlear microphonics but very weak and atypical nerve responses By contrast, damage of the organ of Corti with retrograde degeneration abolishes primarily the cochlear microphonics whereas the compound VIII nerve action potential is barely affected when only the outer hair cells are gone and even a small number of surviving inner hair cells is still compatible with a relatively strong compound action potential of the cochlear nerve

Demonstration of entirely differing structural characteristics of the outer and inner hair cells, their completely differing innervation pattern (Spoendlin, 1969) and their association with two different types of neurons (Spoendlin, 1975), raises the question of the functional role of these different elements With the purpose of elucidating these important questions we produced experimentally serious and (as known from earlier experiments), well defined pathological conditions in cat cochleas by section of the cochlear or VIII nerve or by mechanical and toxic damage of the organ of Corti, with subsequent retrograde degeneration of the cochlear neurons The functional state of the inner ears was measured by electrocochleography (E Co G) before, at different times after the setting of the cochlear damage and immediately before sacrificing the animal

A complete quantitative morphological evaluation of the cochlea was made using the bloc-surface technique (Spoendlin & Brun, 1974) with EM examination of selected parts where surface examination and ordinary light microscopy appeared to be inadequate

Stimulation and recordings were done with the Amplaid-Medelec E Co G apparatus using plain and filtered clicks Cochlear microphonics (CM) and whole nerve action potentials (AP) were always recorded For AP-recordings the CM were eliminated by alternating the polarity of the stimulus

The recording electrode was an insulated steel electrode placed in a small pit made with a diamond drill close to the medial rim of the round window In order to follow the time course of functional deterioration after section of the cochlear nerve, recordings were made immediately before and after the lesion as well as after various time lags, from several hours to as much as 4 months Permanently implanted electrodes proved to pose considerable problems so that we preferred to reopen the bulla for each recording, 2–4 times in each animal, whereby the recording electrode was always placed at the same location in the small pit in the bone made at the first recording medial to the round window A slight inflammatory thickening of the mucosal membrane around the electrode could not be avoided and might have produced a slight conduction deafness in later recordings

With this recording site, normal recordings prior to the lesion showed the typical N_1-N_3 response with usually a pronounced positive wave between (Fig 1 B) The peak to peak amplitude varied between 100 and 160 μV and the latencies were about 1 ms at 100 dB and 3 ms at threshold The threshold for the AP was usually found around 0 dB SPL In the E Co G, the amplitudes of AP s rise slowly in a first phase from threshold to about 50 dB and much

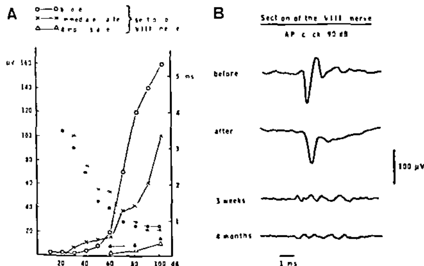


Fig 1 (A) E.Co G with plain clicks in normal cat cochlea and after sectioning of the VIII nerve ——, Amplitudes of AP (µV), ----, latencies (ms) (B) AP's before, immediately after, and 3 weeks or 4 months after sectioning of the VIII nerve. The stimulus is at the beginning of the tracing. The latencies include 2 ms from the distance loudspeaker–ear

faster in a second phase above 50 dB (Fig 1 A) Cochlear microphonics were recorded for 500, 1 000, 2 000 and 4 000 Hz filtered clicks They showed a typical input–output curve with a maximum between 90 and 100 dB SPL Best responses were obtained at 2 000 Hz, probably because this frequency area at the beginning of the second turn was closest to the recording electrode

Sectioning of the cochlear or the entire VIII nerve in the inner acoustic meatus was done through an occipital approach carefully avoiding damage to the labyrinthine blood vessels which were usually identified between cochlear and vestibular nerve

Unchanged normal cochlear microphonics immediately after the nerve lesioning proved an unimpaired blood supply to the inner ear The AP s showed the same threshold and the same latencies In the low intensity range up to 60 dB the response was widened but the amplitude remained unchanged, whereas in the high intensity range the amplitudes were reduced by about 30 % of the normal value before the nerve sectioning This reduction of amplitude was due to the loss of the P_1 wave, whereas N_1 and N_2 remained unchanged (Fig 1 B) The disappearance of P_1 was a consistent finding both after sectioning of the VIII nerve which included the efferents and after selective cochlear nerve sectioning, in which case the efferents were spared as histologically demonstrated when the animal was sacrificed at a later date (Fig 2) This phenomenon can therefore not be due to the interruption of efferent activity as suggested by Fisch & Ruben (1962), as they observed a similar change of AP after sectioning of the VIII nerve

This type of nearly normal AP was still found 24 hours after lesioning, after which time no structural alterations were detectable in the neurons However, after 4 days and in all subsequent recordings up to 4 months following the lesion, normal AP's had completely disappeared, whereas the cochlear microphonics were easily recorded with normal thresholds and even larger amplitudes than in normal animals Instead of normal AP s, very strange, stimulus-related electrical responses were recorded in all these animals The latencies appeared to be very short and did not change much with different stimulus intensities (Fig 1) The recorded waves had maximum amplitudes between 10 and 20 µV and were usually maintained over several ms In many respects they were comparable to CM except that they were irregular and unrelated to the stimulus frequencies and had much smaller amplitudes than the CM of the same ear

These response characteristics were main-

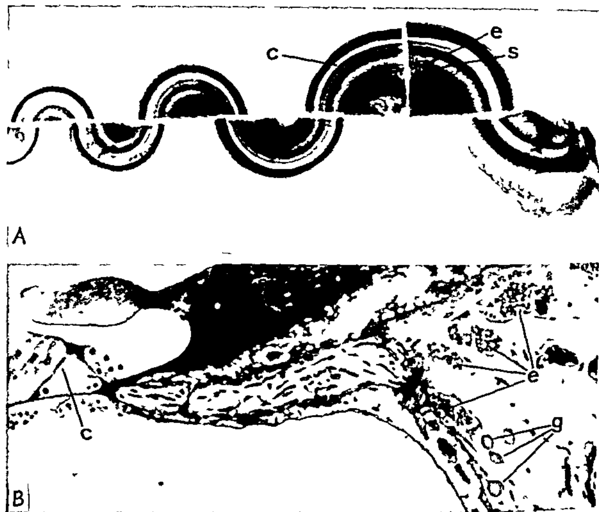


Fig 2 Cat cochlea 4 months after selective sectioning of the cochlear nerve (A) Bloc surface preparation showing an intact organ of Corti (*C*) and almost complete loss of nerve fibres in the osseous spiral lamina (*S*) except the efferent intraganglionic spiral bundles (*e*) (B) Radial section through the upper basal turn from preparation shown in (A) Intact organ of Corti (*C*) empty spiral ganglion with only a few remaining ganglion cells (*G*) and preserved efferent intraganglionic spiral bundles (*e*).

tained during the entire period of nerve degeneration, which became morphologically evident after a few days and was nearly complete after about 2 months After this time only about 5% of neurons remain in the cochlea whereas the organ of Corti shows a normal hair cell population with an entirely normal afferent nerve supply of the outer hair cells but nearly complete loss of the afferent nerve supply of the inner hair cells and, in cases with sectioning of the cochlear and vestibular nerves, also complete loss of the efferent innervation as has been demonstrated in earlier experiments (Spoendlin, 1971) (Fig 2) The remaining 5% of afferent neurons consist mainly of type II neurons and only a few type III neurons, which are modified type I neurons (Spoendlin, 1975) However, only about 2% of myelinated nerve fibres remain as counted in transverse sections through the osseous spiral lamina because most type II spiral ganglion cells have unmyelinated axons

The correlation of these anatomical findings with the E Co G recordings confirms that CM are not affected by sectioning and degeneration of the VIII nerve (Kiang & Peake, 1960, Ruben et al, 1962) as long as the blood supply and

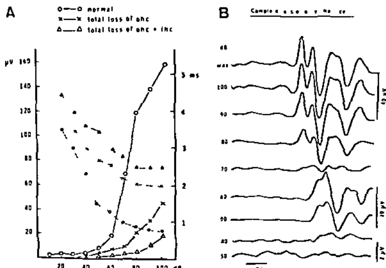


Fig 3 (A) E Co G after severe damage to the organ of Corti (B) AP s of the cochlea shown in Fig 4

therefore the hair cell population remains intact The frequently observed enhancement of the CM after sectioning of the cochlear and the VIII nerve might be due to the fact that they are not interfered with so much by potentials of neural origin The strange potentials registered with AP-recordings after degeneration of the VIII nerve might be a product of incorrectly cancelled CM, though this seems unlikely as one would expect them to change with stimulus frequency On the other hand they could be related to the intact afferent nerve supply of the outer hair cells which consists of a great number of outer spiral fibres (about 100 at any one place in the organ of Corti with their numerous nerve endings at the outer hair cells) and the tunnel crossing basilar fibres and which is associated with the few remaining type II and III cochlear neurons We must assume that these numerous peripheral fibres which represent the afferent nerve supply of the outer hair cells produce some electrical activity The complex and changing form of these recorded responses probably expresses highly dissociated and poorly synchronized activities and the short latencies indicate a highly peripheral origin, peripheral to the initial segment of the neurons The positive W potential described by Ruben et al (1962) with direct recordings after sectioning of the VIII nerve might be contained in these potentials we recorded with E Co G Their W-potential has the same order of magnitude but greater and stimulus-dependent latencies

An entirely different picture results if secondary nerve degeneration is the consequence of destruction of the organ of Corti by locally applied ototoxic antibiotics or direct mechanical damage

A single injection of 500 mg Neomycin into the bulla of the cat usually leads to a complete loss of inner and outer hair cells except for a few remaining abnormal inner hair cells After one year 80 % to 90 % of neurons have undergone retrograde degeneration and have disappeared The great majority of the surviving neurons are of type I or III and a corresponding number of myelinated neurons is found in the osseous spiral lamina In contrast to the degeneration following sectioning of the VIII nerve, the afferent nerve supply of the outer hair cells disappears or is severely impaired

In the E Co G recordings the CM are entirely missing, but weak yet clear AP s with a slightly modified form and a greater latency can still be recorded The fact that AP s can still be recorded despite a total loss of outer and subtotal loss of inner hair cells would indicate that nerve fibres can be acoustically stimulated to a certain extent in the absence of normal hair cells The possibility that these potentials are

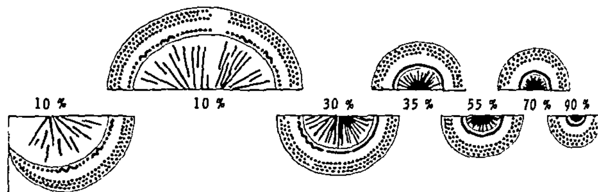


Fig 4 Cochleographic reconstructions of a cat cochlea 1 year after mechanical damage in lower basal turn Blank area site of lesion Dots missing hair cells but intact support structures Full heavy lines intact hair cells. Numbers indicate the remaining myelinated nerve fibres in the osseous spiral lamina as percent of normal

activities from the contralateral ear is unlikely as we did not find them in experiments with VIII nerve sectioning For contralateral brainstem potentials they are probably too large and in the low intensity range their latencies are not much greater than normal (Fig 3A)

When the organ of Corti was mechanically destroyed in the lower basal turn with a small needle through the round window or through an opening in the scala tympani from the inner acoustic meatus, we found in 4 animals a complete but rather selective loss of outer hair cells, whereas the inner hair cells remained morphologically normal in the greater part of the cochlea (Fig 4) This appeared to be the best way for selective destruction of outer hair cells in cats, possibly by contamination of the organ of Corti with endolymph to which the outer hair cells are much more exposed and therefore probably more susceptible than the inner hair cells In spite of the full presence of normal inner hair cells, the number of unmyelinated nerve fibres was frequently reduced by as much as 70% which shows that retrograde nerve degeneration is not necessarily a consequence of inner hair cell loss, as already mentioned earlier (Spoendlin, 1975)

Despite the total loss of outer hair cells, a reduced number of inner hair cells and a 70% reduction of cochlear nerve fibres, relatively good AP's with a slightly elevated threshold at 30 dB SPL and a maximum amplitude of 40 μV could be recorded in these animals—but no CM (Fig 3) The latencies of the AP s were increased by 1 ms for higher stimulus intensities but within normal limits in the lower stimulus intensity range The form of the response was modified by the appearance of a strong initial positive wave The input–output function of the AP's tended to lose its two-segmented stage (Fig 3A) Whereas the threshold is only slightly elevated, the maximum output at 100 dB is greatly reduced (by about 70%) The main functional impairment in these pathological conditions therefore seems to be proportional to the reduction of cochlear nerve fibres, i e 70% in the example cited above

The complete lack of CU in the absence of outer hair cells but normal inner hair cells and supporting structures illustrates that the outer hair cells are the main source of CM and that CM do not reflect activity of the inner hair cells provided the excitation mechanism of the inner hair cells is not disturbed by the loss of outer hair cells

The correlation of E Co G and cochlear pathology in these experiments leads to the following conclusion degeneration of the VIII nerve does not affect the CM

No CM are recorded when all outer hair cells are gone and only inner hair cells are present

Some acoustical stimulation of nerve fibres still seems possible when all hair cells are missing except a few severely altered abnormal inner hair cells

After sectioning and degeneration of the VIII

nerve, where the afferent nerve supply of the outer hair cells remains selectively intact, a small stimulus-related atypical potential can be recorded and might originate in the afferent nerve supply of the outer hair cells

There is a principal difference between secondary nerve degeneration following sectioning of the VIII nerve and following damage to the organ of Corti After section of the VIII nerve only extremely few myelinated nerve fibres but an intact afferent nerve supply to the outer hair cell remains and no normal AP's can be recorded, whereas after destruction of the organ of Corti a greater number of myelinated nerve fibres remain but the afferent nerve supply of the outer hair cells has disappeared or is highly impaired and some AP's can still be recorded proportional to the remaining nerve fibres

RESUMÉ

Des al ...

piète des potentiels microphoniques pendant que les po-
ten ...

ZUSAMMENFASSUNG

Bei experimentell geschädigten Innenohren wurden die durch bloc-surface-technique und Elektronenmikroskopie quantitativ erfassten strukturellen Veränderungen

...

nes mit retrograder Degeneration führt andererseits zu einem primären Verlust der Mikrophonpotentiale, wo-

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DISCUSSION

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BRAIN STEM ELECTRIC RESPONSE AUDIOMETRY (BSERA)

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Abstract Brain stem electric responses, recorded with external electrodes on vertex and ear lobes, *are excellent* for audiometry of young children The vertex positive wave with latency of 6 to 9 msec resembles closely the action potential of the auditory nerve with the same high intensity short latency component and low intensity long latency component Thresholds are reliable with filtered clicks at 1 000 Hz and higher Practical advantages and theoretical limitations are summarized

Electric response audiometry, based on averaged auditory responses, is now a familiar technique in many hearing clinics Its major usefulness is for testing the hearing of young or otherwise uncooperative children There are good hopes also that one form of ERA, namely the electrocochleogram (ECochG), may yield unique diagnostic information concerning the inner ear and auditory nerve I have recently reviewed the entire subject in a monograph supplement (no 28) of *The Annals of Otology, Rhinology and Laryngology* The present paper is published as a footnote to that monograph in order to add certain experimental details and to emphasize the suitability of this form of audiometry for young children

Any form of ERA for very young or hyperactive or emotionally disturbed children requires either sedation or general anaesthesia It is this requirement that makes the familiar cortical ERA (slow responses) unsatisfactory because in sleep or under sedation the thresholds of these responses are elevated and their identification is less reliable than in the waking state This practically reduces the clinical alternatives to the "middle" (cortical) responses the brain stem responses, or the electrocochleogram These three are not significantly modified by sedation

The cortical responses have a great theoretical advantage at low frequenies (500 Hz and below) and will probably become the method of choice for low frequency thresholds On the other hand, at 1 000 Hz and above, the ECochG and BSER both show such distinctive sharp waves, with clearly defined latencies and low thresholds, that they will almost certainly prove to be more reliable and sensitive at the middle and higher frequencies than the later cortical responses

The use of properly filtered clicks (tone pips) gives enough acoustic selectivity at 1 000 Hz and higher to yield a satisfactory clinical audiogram, although further validation with pathological ears is still very desirable (The question of proper acoustic stimuli and acoustic selectivity is treated at length in my monograph)

The case for the BSER as opposed to ECochG rests primarily on the external placement of the electrodes, on vertex and earlobes Simple sedation (for example by secobarbital) is quite sufficient This procedure can be carried out in a hearing clinic under medical supervision, and it is not necessary to have an anaesthetist to administer a general anaesthetic or an otologist to place a transtympanic electrode on the promontory, as is usual for ECochG

One undisputed advantage of ECochG is that the response is strictly homolateral Conse-

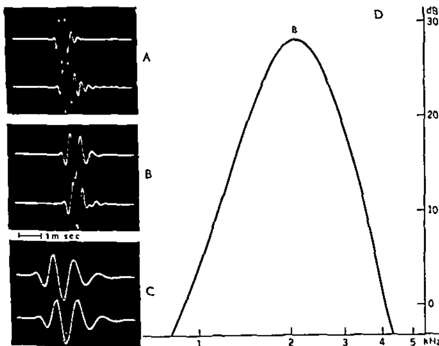


Fig 1A D (*A, B, C*) Oscillograms of tone pip output from a commercial ERA instrument at 1 000, 2 000 and 4 000 Hz respectively Upper trace, electric output, lower trace, acoustic output, through earphone on a 6 cc coupler (*D*) Envelope of acoustic energy spectrum of the electrical output at 2 000 Hz shown in B The signals were formed by passing one sine wave through a band pass filter set to the same frequency

quently stimuli may be delivered from a loudspeaker and masking of the contralateral ear is not necessary

Another advantage is that, with adequate technical precautions, the cochlear microphonic (CM), generated chiefly by the external hair cells of the basal turn, may be recorded in addition to the action potential of the auditory nerve This may prove to be useful in adults when the differential diagnosis is sense-organ impairment versus acoustic neurinoma In young children, however, this question rarely arises and ECochG can be held in reserve for such possible situations

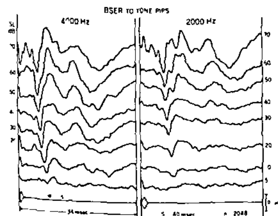


Fig 2 Brain stem electric response P_6 of a young adult in natural sleep to tone pips at 4 000 Hz (*left*) and 2 000 Hz (*right*) Electrodes on vertex and earlobe Upward indicates vertex more negative Sensation level of stimulus at right and left. Timing and approximate envelope of electric tone pips to earphones shown at bottom.

Until recently it seemed that ECochG might have another advantage in the systematic changes of latency and amplitude of its neural response with the intensity of the stimulus There seem to be two populations of auditory units, one with a high threshold (≈ 50 dB HL) and a short, stable latency, the other with its threshold very near the behavioral threshold and a latency that is prolonged by about 3 msec at threshold

One of my major points today is that the most prominent wave (P_6) of the normal brain stem response, the one sometimes called "Jewett V", with positive peak at about 6 msec

for a high frequency stimulus at 60 dB HL, follows very accurately the first neural wave (N_1) of ECochG with a latency difference of almost exactly 4 0 msec This P_6 wave shows the same low threshold with prolonged latency as N_1 AP in ECochG Whatever the physiological interpretation and the diagnostic significance of the "two populations" turn out to be, it is very probably that BSER will share with ECochG the advantages of revealing them separately

Experiments using unfiltered clicks or a 2 000 Hz click (Elberling) seemed to relate the short-latency responses chiefly to the nerve fibers innervating the basal turn, but we have found that the two sets of responses are still present in BSER when the stimuli are 4 000 Hz filtered tone pips Of course there is some acoustic spread to higher and lower frequencies with brief (tone pip) stimuli, but the difference in traveling wave delay due to it is not enough to account for the very long latencies near threshold at 2 000 and 4 000 Hz We do see, however, a systematic difference between the latencies for these two frequencies, just as we should expect from their different traveling wave delays The original guess that the two populations

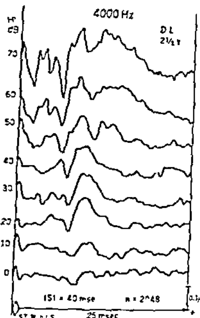


Fig 4 Brain stem electric responses P_6 of a 2½-year-old child under secobarbital sedation. Similar to Fig. 2 except for shorter analysis period and a higher low-frequency cut-off (≈ 150 Hz) in the recording system.

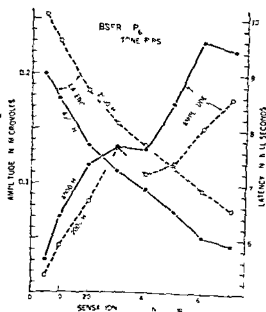


Fig 3 Latency and amplitudes of the responses shown in Fig. 2, plotted as functions of sensation level The latency for 2 000 Hz is systematically longer than for 4 000 Hz by about 0 8 msec at each sensation level. The amplitude is less for 2 000 Hz at most levels Amplitude of P_6 is average of the differences between P_6 and the negative peaks about 1.5 msec before and 3 0 msec after it.

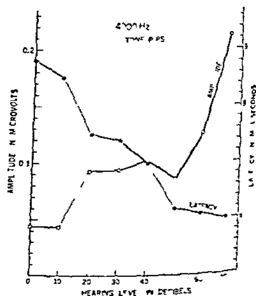


Fig 5 Latency and amplitudes of the responses shown in Fig. 2, plotted as functions of hearing level (ISO). Amplitude of P_6 is average of the differences between P_6 and the negative peaks about 1.25 msec before and after it.

of responses are initiated by inner and outer hair cells respectively may still prove to be correct We await with great interest both the results of animal experiments with ototoxic drugs and acoustic trauma and also careful clinical validation on pathological ears with several frequencies of tone pips and good behavioral audiometry We already know that the low-threshold population appears to be absent in cases with sense organ impairment and recruitment of loudness We must rest on empirical evidence here, both for BSER and ECochG, because the theoretical situation with respect to the nature of the two populations is still too confused to give a sound basis for interpretations

RÉSUME

Les potentials electriques du tronc cérebral, enrigistres a partir des electrodes situees sur le vertex et le lobe de l oreille font bien possible l'audiométrie chez les enfants L'onde positif au vertex, avec une latence de 6 à 9 mec, ressemble au potential d action du nerf auditif avec des composants semblables, celui de latence courte et d intensite haute, et un autre de latence longue et d intensite faible Les seuils sont assez exacts quand on emploit des clics filtres de 1 000 Hz et audessus. On présente les avantages pratiques et les limitations théoretiques

ZUSAMMENFASSUNG

Elektrische Antworten des Hirnstammes, mittels äußerer Elektroden vom Scheitel und Ohrläppchen abgeleitet, sind vorzuglich fur die Audiometrie von Kleinkindern geeignet Die scheitelpositive Welle mit einer Latenzzeit von 6–9 Millisekunden ist dem Aktionspotential des Hornerven sehr ahnlich, mit den gleichen Komponenten „hohe Intensitat, kurze Latenz" und „niedrige Intensitat, lange Latenz" Verlaßliche Horschwellen werden mit Hilfe von bei 1 000 Hz und daruber gefilterten Clicks erreicht

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Any member of the Collegium ORLAS may receive a copy of this monograph gratis on request directed to the Beltone Institute for Hearing Research, 4201 West Victoria Street, Chicago, Illinois 60646

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DISCUSSION

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Acta Otolaryngol 83 140–145, 1977

TYMPANOMETRY AND ACOUSTIC IMPEDANCE

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Abstract In recordings of the acoustic impedance of the middle ear [illegible]

tone frequency close to the resonance frequency of the middle ear, characteristic tympanometric patterns can be demonstrated in certain middle ear lesions The shapes of the curves are discussed in relation to the behaviour of the different components of the middle ear impedance

Tympanometry is an audiological test based on impedance principles described by Metz (1946) and further outlined by Thomsen (1955), Anderson (1956), Terkildsen & Thomsen (1959) In the last few years it has gained popularity due to works by Brooks (1968, 1969), Feldman (1969), Jerger (1970) and Liden et al (1970)

Tympanometry provides us with information about how acoustic energy flows through the middle ear as the air pressure is varied across he tympanic membrane Thus, tympanometry can be used for assessment of the mobility of the tympanic membrane, the dynamics of the ossicular chain, and the middle ear pressure The air cushion of the tympanic cavity and the eustachian tube function can also be evaluated Consequently the method is of great value in the clinical diagnosis of different middle ear lesions Tympanometry is performed by generating a probe tone in the external ear canal and recording the change in the sound pressure level of the reflected probe tone while altering automatically the air pressure in the ear canal The recorded change of the sound pressure level indicates the change of impedance of the ear drum and the middle ear

The interpretation of tympanograms is made by analysing the three essential tympanometric features pressure, amplitude and shape, which all are influenced by the transmission factors at the ear drum In this paper only amplitude and shape will be discussed The amplitude of the tympanogram represents the change in compliance (the inverse of stiffness) of the tympanic membrane, between its most tight position and its loosest position This difference can be expressed in acoustic millimhos, in equivalent cc of air volume, or in equivalent air volume relative to the volume of the external ear, in dB Because the reflected sound pressure is not exactly in phase with the applied probe tone particularly at high frequencies, it is sometimes informative to express the tympanogram in terms of a complex acoustic admittance vector $Y_a = G_a + jB_a$ with a conductance G_a and a susceptance B_a The magnitude of the admittance can be found as

$$|Y_a| = (G_a^2 + B_a^2)^{1/2}$$

Impedance is the reciprocal of admittance Thus, $Z_a = 1/Y_a$, Z_a has two components as well a resistance (R_a), and a reactance (X_a) $Z_a = R_a + jX_a$, and the magnitude of Z_a is

$$|Z_a| = (R_a^2 + X_a^2)^{1/2}$$

Unfortunately, there is no commercially available bridge for direct measurement of Z_a in terms of its components However, the Grason

This study has been supported by the Swedish Medical Research Council (grant no B76-17X 133 10) the Swedish Labour Environmental Protection Fund (grant [illegible]

Stadler otoadmittance meter does, as the name implies, measure the conductance and susceptance, from which the admittance can be calculated

Stiff middle ear systems such as in otosclerosis restrict the amplitude of the peak of the tympanogram below the normal range Ossicular interruption as well as ear drum abnormality exaggerate the amplitude (Fig 1) Comparison of the amplitude to the normal range determines whether the static acoustic impedance is normal, low or high

The shape of the tympanogram is the second parameter of diagnostic interest Until recently tympanometry has mostly been performed with 220 Hz as the probe tone frequency Three types of curves are well established The most common is the V shaped curve with normal amplitude of the peak indicating good mobility of the middle ear system and maximum flow of acoustic energy at atmospheric pressure A peak at negative pressure implies underpressure in the middle ear, indicating eustachian tube blockage The third type shows a flat or relatively flat curve commonly found in serous otitis, chronic adhesive otitis or massive ossicular fixation

Liden et al (1970, 1974) pointed out that with a high frequency probe tone (800 Hz) two more characteristic types of tympanograms could be recognized These proved especially helpful in finding cases with ossicular interruption and/or ear drum abnormality such as atrophic scarring The former pathology gives rise to tympanograms *showing broad and deep undulating* notching, and the latter shows W-curves with sharp double peaks (Fig 2) In static acoustic impedance measurements the presence of a

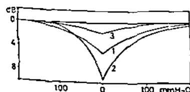


Fig 1 Tympanograms illustrating (1) normal middle ear (2) ossicular interruption, and (3) otosclerosis. Probe tone 220 Hz.

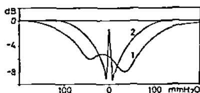


Fig 2 Tympanogram with probe tone 800 Hz, (1) ossicular interruption (2) ear drum abnormality (scar)

healed perforation on the drum may mask the occurrence of stapes ankylosis by turning high impedance scores into low ones (Feldman, 1974) Thus, from a diagnostic viewpoint it is important to make a high-frequency tympanogram before the measurement of the static acoustic impedance With this exception the W-tympanogram is of minor clinical interest, because it can also be found in subjects with normal looking but very mobile drums (Colletti, 1976, Vanhuyse et al , 1975) A broad, smoothly undulating tympanogram, changing its directions with induced negative and positive air-pressure changes is easy to separate from the sharp double peak tympanogram and is important as it indicates interruption of the ossicular chain

Tympanometry utilizing higher frequency probe tones makes it possible to analyse the stiffness-mass relationship of the ear A discontinuity of the ossicular chain lowers the resonant frequency of the middle ear and makes the transmission system mass controlled Externally applied, negative or positive air pressure changes in the ear canal increase the stiffness factor and, *if sufficiently close to the resonant frequency*, shifts the direction of the tympanometric curve

According to Møller (1965) the acoustic resistance in the normal ear comes mainly from the cochlea The resistive component is diminished for negative air pressure in the ear canal indicating partial decoupling of the cochlea A lowered resistance thus is to be expected in cases with interruptions of the incudostapedial joint when the cochlea is disconnected On the other hand it is well known that the compliance is greatly increased in ossicular interruptions Thus it seemed to be of interest to

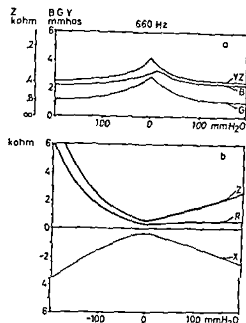


Fig 3 Normal subject Probe tone 660 Hz. (*a*) *G* and *B* curves *Y* and *Z* calculated (*b*) Impedance values in the plane of the drum

determine which of the impedance components is most affected, and which one provides most diagnostic information in cases with separation of the ossicular chain

Our 800 Hz acoustic bridge indicates only changes in the magnitude of the acoustic impedance and thus was replaced by the Grason-Stadler 1720 otoadmittance meter This one employs 220 and 660 Hz as probe tones and both conductance and susceptance, i e "G" and "B" curves can be recorded With low frequency probe tones the shape of the tympanogram in ossicular disruption is mostly normal, although the amplitude may be exaggerated The 660 Hz "B" tympanogram, however, is often distorted in a similar way as with the 800 Hz probe tone

MATERIAL

Four non successful stapedectomies with hearing loss for speech between 57–73 dB HL were investigated Surgery performed after the examination revealed complete interruption of the ossicular chain due to dislocation of the steel wire prosthesis 8 subjects with otosclerosis and completely normal-looking ear drums and 3 with otosclerosis showing small atrophic scars on the drums were investigated before surgery Furthermore, 8 normal-hearing persons with hearing equal or better than 15 dB ISO in the frequency range 250–8 000 Hz were included in the investigation

EQUIPMENT

The Grason Stadler model 1720 otoadmittance meter was used for determining the tympanograms Conductance and susceptance for 220 and 660 Hz probe tones were recorded In order to convert these tympanograms into resistance, reactance, and impedance in the plane of the drum the otoadmittance meter was directly connected to a PDP 8e computer

RESULTS

The measured conductance and susceptance (G_a and B_a) curves and the calculated admittance (Y_a) curve for 660 Hz probe tone of a subject with normal hearing and normal tympanic membranes as well as the corresponding impedance curves in the plane of the drum are shown in Fig 3 Mean values of G_a, B_a, Y_a ,and Z_a for the different groups of subjects are given in Table I For comparison, some other results for normal ears and ears with abnormalities (Feldman, 1974), and for otosclerosis (Zwislocki & Feldman, 1970) and ossicular disruption (Lilly 1973) are given in the same table Fig 4 demonstrates G_a, B_a and Y_a tympanograms for 660 Hz and corresponding resistance, reactance and impedance curves in the plane of the drum for a typical case with complete ossicular interruption

Measurements of absolute (static) acoustic impedance is especially valuable when combined with high-frequency tympanometry Normal otoscopy, slight conductive loss, and normal shaped tympanogram with high acoustic impedance indicates incipient otosclerosis Low acoustic impedance is common in ossicular separation but also in cases with scarring of the

Table I *Admittance and impedance values in different groups of hearing losses*

Tympanogram	Normal ears		Otosclerosis		Interruption of ossicular chain		Abnormality of drum	
	Mean values		Mean values	Median 80 % range			Otosclerosis	
	This paper	Median, 80 % range Feldman, 1974	This paper	Feldman & Zwislocki, 1970	This paper	Lilly, 1973	This paper	Normal hearing Feldman 1974
Number	8	100	8	24	4	12	3	29
G_a 220	0 03	0 15 (0 05–0 35)	0 08		0 55		0 48	0 5 (0 2-0 85)
B_a 220	0 57	0 5 (0 3–0 75)	0 28		2 11		1 63	1 5 (1 0–2 5)
Y_a 220	0 62	0 52 (0 33–0 85)	0 30		2 18		1 70	1 6 (0 95–2 6)
R_a 220/250	693	633[a]	705	520 (322 1 190)	138	120 (63–229)	172	
X_a 220/250	1 640	1 563[a]	3 590	3 530 (2 332 5 883)	542	609 (369 950)	661	
Z_a 220/250	1 780	1 856 (1 124–3 024)	3 710	3 568 (2 354–6 002)	559	621 (374–977)	683	625 (380–1 059)
G_a 660	1.39	1 95 (1 0–4 2)	0 33		6 0		5 1	6 8 (4.25–10 1)
B_a 660	1 24	1 3 (0 9–2 45)	0 84		2.3		2 53	2.7 (0 6–4 55)
Y_a 660	1 88	2 43 (1 38–4 5)	0 90		6 43		5 78	7 9 (4 55–11 1)
R_a 660/750	464	438[a]	404	295 (218–646)	152		162	
X_a 660/750	417	326[a]	1 130	980 (614–1 471)	62.0		95	
Z_a 660/750	625	409 (220–715)	1 210	1 023 (652 1 607)	165		191	135 (90–220)

[a] Feldman, A S & Williams, P S , 1976

tympanic membrane High frequency tympanometry is needed for the differential diagnosis The notching pattern associated with ossicular disruption is broad and undulating and different from the sharp double peak pattern seen in eardrum abnormality

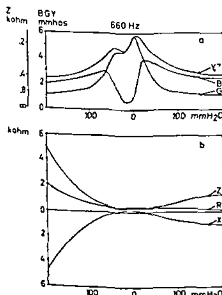


Fig 4 Ossicular interruption (*a*) *G* and *B* curves *Y* and *Z* calculated (*b*) Corresponding impedance values in the plane of the drum

DISCUSSION

The primary purpose of this paper was to determine which of the impedance components is most affected, and which one provides most diagnostic information, in cases with separation of the ossicular chain In order to ascertain this, the results of our relatively small number of subjects in different groups were compared to the results of measurements on larger groups Our results, as seen in Table I, show good agreement with the bigger groups

As expected, the acoustic impedance employing both probe frequencies was very low in cases with complete ossicular separation compared to the normal middle ear At 220 Hz the acoustic resistance and reactance was 20 % and 33 % respectively of what was found in normal subjects At 660 Hz the corresponding values were 34 % and 15 %

The acoustic resistance is almost independent of frequency A lowering of the resistive component as in cases with complete ossicular separation will generally not distort the regular tympanometric pattern as this component cannot become negative It may, however, exaggerate the amplitude of the peak

In subjects having either otosclerosis, normal middle ear, or ossicular separation the reactance component in the low frequency range is usually negative, i e the transmission system of the middle ear is stiffness controlled This indicates that the probe frequency is below the resonant frequency of the middle ear Notching of the low frequency tympanogram is also very rare A mass controlled middle ear transmission system is common in normal subjects with large pneumatization in the frequency range 500–1 000 Hz (Lilly, 1973) The resonant frequency of the middle ear is lowered due to ossicular separation Low values of acoustic reactance are expected in cases with ossicular disruption, and it equals zero when the probe frequency and the resonant frequency of the middle ear coincide In Fig 4 the acoustic reactance curve is positive close to ambient air pressure but turns negative as the air pressure in the ear canal changes in either negative or positive direction There is a change from mass to stiffness control when the reactance curve passes through the resonant point and turns from positive to negative This phase shift is displayed in the tympanogram as changing of direction As a rule, the larger the distance between the zero-crossings of the reactance curve the more undulating and broad the tympanogram will be Small differences between the zero crossings will give sharp W tympanograms

Finally we can conclude that in ossicular interruption both resistance and reactance components of impedance are diminished significantly compared with the normal population This is true for both low and high frequency probe tones From a diagnostic viewpoint, however, the irregular shape found only in high frequency tympanograms due to the change in sign in acoustic reactance close to resonance, has the greatest importance

Mrs Evy Nilsson has given valuable help collecting the material

RÉSUME

Lors de tympanométrie on fait varier le composant capacitif de l impedance d l oreille moyenne en modifiant la pression dans le conduit auditif Si la fréquence de relevé est choisie de sorte qu elle se trouve suffisamment pres de la frequence de resonance de l oreille moyenne on obtient un tympanogramme caractéristique lors de rupture dans la chaine des osselets de l oreille

ZUSAMMENFASSUNG

Bei den Aufnahmen der akustischen Impedanz des Mittelohres (Tympanometrie) beeinflußt der Wechsel des Luftdruckes im Gehorgang meistens den Kapazitätskomponenten der akustischen Impedanz Wenn die Frequenz des Testtones so gewahlt wird daß diese in der Nahe der Resonanzfrequenz des Mittelohres liegt konnen charakteristische tympanometrische Muster bei gewissen Mittelohrstorungen gezeigt werden Die Form der Kurven werden im Verhaltnis zu dem Verhalten der verschiedenen Komponenten der Impedanz des Mittelohres diskutiert

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DISCUSSION

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OXYGEN RESERVE AND AUTOREGULATION IN THE COCHLEA

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Abstract Endolymphatic O_2-concentration was measured in the guinea pig cochlea with a microelectrode inserted thro[illegible] hypoxia produced by lowering the pO_2 of the inspired air Persistence of a normal endolymphatic O_2 level following the fall in arterial pO_2 indicated an O_2 reserve and an autoregulation of the cochlear circulation

The problem of maintaining blood flow through the capillaries of the membranous labyrinth is a most important one if we are to prevent or reverse certain forms of sensorineural deafness Occlusion of any one of the separate capillary areas, while affecting different aspects of the complicated transduction process, can result in decreased function and deafness As a consequence, a question that has plagued otologists concerns a possible mechanism for keeping open or opening up capillary flow when cochlear ischemia is suspected

The blood supply to the ear is similar to that of the brain and one would expect that both organs would demonstrate the same blood flow characteristics One of the extensively studied characteristics of cerebral blood flow is that of autoregulation, and Perlman & Yamada (1967) have demonstrated that this phenomenon occurs also in strial blood flow

Autoregulation is variously defined, but, in general, it refers to the ability of a peripheral vascular bed to maintain a constant blood flow over a limited range of arterial perfusion pressure The capillary flow in brain and membranous labyrinth is peculiarly resistant to neurogenic control, while responding more readily to local metabolic demands Thus the homeostatic condition is maintained by a peripheral vascular response to perfusion pressure changes and variations in the products of metabolism

Perlman & Yamada (1967) demonstrated autoregulation in strial blood flow through motion picture analysis Despite a sustained reduction in carotid pressure, strial flow was seen to return to normal flow after an initial decrease However, the method they used was incapable of detecting continuous changes in strial blood flow and of monitoring any other capillary area

Because the various capillary areas of the membranous labyrinth are serving different tissues for different functions, and altering the arterial flow to the different sets of capillaries is most difficult, we have taken the different approach of determining the constancy of oxygen provision to the fluid spaces and tissue areas Respiratory air to the animal is cut off and the ability of the peripheral blood flow to maintain constancy is determined by recording the difference between delay of oxygen concentration depletion in the tissue and that in the arterial blood from the heart

When oxygen supplied to an anesthetized animal is reduced either by respiratory shut off or by changes in the air mixture, there is a delay before an effect is observed in the function of the organ of Corti as manifest in its various electrical potentials This was observed

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in an early study of the effects of anoxia (Wever et al, 1949) and we have recently used the delay measures to determine the role of the spiral capillaries along the basilar membrane border of the osseous spiral lamina (Lawrence & Nuttall, 1972)

Misrahy et al (1958) were among the very few who ever measured oxygen tension in the endolymph and attempted, following trachea clamp, to correlate the delay difference between oxygen depletion and decrease in the various potentials They concluded, erroneously, that the endolymph oxygen supplies the hair cells

Tsunoo & Perlman (1969) timed the delay in fall of perilymph (scala vestibuli of basal turn) oxygen and of cochlear a c potential following occlusion of arterial blood supply and interpreted the rapid recovery and overshoot of oxygen tension as indicative of autoregulation

Our purpose, in the experiments reported here, was to determine the lag in the onset of fall in oxygen concentration in the fluid spaces and capillary areas of the membranous labyrinth following the initial fall in oxygen concentration in arterial oxygen measured by a microelectrode in the carotid artery after shut-off of respiratory air Admittedly, shutting off respiratory air is a drastic procedure, as is clamping the trachea or aorta, but the ability of the labyrinthine microcirculation to provide the fluid spaces and tissues with oxygen for only a few additional seconds indicates that local factors are operating Under more subtle conditions of isolated localized ischemia, this autoregulation may be very important and if the factors that are maintaining this peripheral capillary flow can be determined, then means of preventing or reversing ischemic deafness may be at hand

When oxygen supply to the animal is reduced by interrupting respiration, and a measure of oxygen content of arterial blood at the heart is taken as a starting point, there are several factors that could cause a delay in measurable oxygen reduction in fluids of an extracellular space in proximity to a capillary network Among these are the transit time for the oxygen-depleted erythrocytes to pass from heart to capillary area, the diffusion time of oxygen passing from a capillary through fluid to the measuring electrode, and changes in the volume flow through the capillary network produced as a reaction to the low oxygen state or perhaps perfusion pressure (autoregulation)

The experiments reported here involve a measure of each of these factors and a determination of the ability of the microcirculation to maintain cochlear function in the face of small variations in arterial oxygen

METHOD

The methods of animal preparation and oxygen concentration determination have been described in an earlier paper (Lawrence et al, 1975), in which, along with the measurement of various cochlear potentials, we determined the delay times of oxygen depletion after shut-off of respiratory air and of oxygen increase after the restoration of respiratory air, by recording from oxygen sensitive electrodes placed in the tunnel-fluid space and in the scala media We have now added a similar determination for three more areas arterial blood in the left common carotid, the fluid space of the scala vestibuli, and within the capillary network of the stria vascularis

The areas from which these recordings were made and the blood supply to the membranous labyrinth are illustrated in Fig 1 The carotid artery was exposed along with the trachea during the ventral approach to the left bulla of the guinea pig A cannula was tied into the trachea to provide artificial respiration and an oxygen-sensitive electrode inserted into the artery

Oxygen recordings were made from the perilymph of the scala vestibuli by insertion of an electrode through a small opening in the capsule bone of the basal turn of the cochlea

Recordings from the stria vascularis were made by exposing the spiral ligament of the second turn The placement of the electrode was guided visually through a microscope, and oxy-

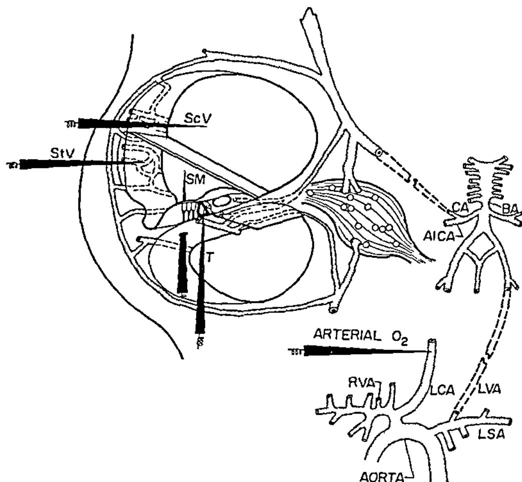


Fig 1 Schematic representation of blood supply to the membranous labyrinth and the placement of oxygen-sensitive electrodes for this study *RVA* right vertebral artery, *LCA* left carotid artery, *LSA* left subclavian tery, *LVA* left vertebral artery, *BA* basilar artery, *CA* cochlear artery, *AICA* anterior inferior cerebellar artery, *T* tunnel electrode, *SM* scala media electrode, *StV* stria vascularis electrode, *ScV* scala vestibuli electrode

gen current monitored as the electrode was advanced The cochlear a c potential was also recorded for a frequency of 500 Hz presented through a tube in the external meatus As the electrode was advanced through the spiral ligament, penetration into the stria vascularis could be determined by a sudden rise in oxygen current If the eletrode penetrated through the stria vascularis entering the scala media, oxygen current decreased and the a c potential would increase

The oxygen within the fluid of the tunnel of Corti was recorded by insertion of an electrode after removing a wedge of bone from the border of the round window, thus exposing the basilar membrane and osseous spiral lamina as described in our previous paper (Lawrence et al, 1975) Usually another electrode was passed through Claudius' cells into the scala media for the recording of oxygen concentration in the endolymph, although in some instances scala media recordings were made from an electrode passed through the stria vascularis

The output from each oxygen current meter was traced on a six-channel graphic recorder and delay times read from the curves Similar recordings were grouped and the mean and standard deviation calculated Forty-three animals were used in addition to those of the previous study and the numbers of separate recordings are indicated in the tables

Table I *Delay times from shutting off respiratory air to decline in oxygen of carotid, tunnel fluid (TUN), stria vascularis (Stria), scala media (SM), and scala vestibuli (SV)*

	Carotid	TUN (Lawrence et al., 1975)	Stria	SM (Lawrence et al., 1975)	SV
No. of runs	40	52	23	52	18
Mean (sec)	4.8	12.1	9.6	14.4	16.2
Std dev (sec)	1.1	3.6	2.2	4.8	2.1

RESULTS

A Delay times

Timing of events from the moment respiratory air is turned off or on is not a very accurate procedure, as the amount of residual air in the lungs and respirator tubing is an unknown quantity. In order to obtain more precise timing, oxygen depletion or return in arterial blood direct from the heart was determined by placing an oxygen sensitive electrode in the left common carotid artery. Although, as Fig. 1 shows, it is the vertebral arteries that supply the inner ears, the carotid is more accessible in our surgical exposure and gives a good representation of oxygenated blood from the heart.

The first determination was of the time taken for the oxygen-depleted blood to pass from the heart to the capillary areas and fluid spaces of the inner ear. The data collected have been combined with those obtained earlier (Lawrence et al., 1975).

The data in Table I display the delay times in seconds, from the moment the respiratory air was turned off to the first noticeable oxygen decrease in left common carotid artery near the heart, in the tunnel fluid, in the capillary bed of the stria vascularis, in the endolymph of the scala media, and in the perilymph of the scala vestibuli.

Once the oxygen level of the blood and tissues has been brought down to a fairly low level, a new set of factors is no doubt introduced, as air is returned to the lungs and the reoxygenation of the blood begins. This is reflected in entirely different delay times as the respiratory air is returned to the animal.

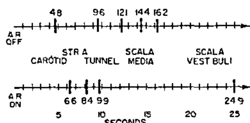


Fig. 2. Plot of the lag times to first detection of oxygen depletion at a microelectrode in the indicated region after interruption of respiratory air and plot of first detection of oxygen increase following restoration of respiratory air.

The data in Table II display the lag, in seconds, between restoration of respiratory air and the first noticeable oxygen increase in left common carotid artery near the heart, in the tunnel fluid, in the capillary bed of the stria vascularis, in the endolymph of the scala media, and in the perilymph of the scala vestibuli.

The lag times between air off and air on are presented comparatively in Fig. 2. Rather marked differences are observed among the off-on lag times. For later calculations the carotid oxygen is taken as the point of departure, which eliminates an obvious factor illustrated by the 1.8 sec longer lag for carotid oxygen to increase after the air is restored than for carotid oxygen to start a decline after the air is turned off. The difference probably represents the time taken to refill the respirator tubing and lungs when the air is turned on.

Table II *Delay times from turning on respiratory air to increase in oxygen of carotid, tunnel fluid (TUN), stria vascularis (Stria), scala media (SM), and scala vestibuli (SV)*

	Carotid	TUN (Lawrence et al., 1975)	Stria	SM (Lawrence et al., 1975)	SV
No. of runs	40	11	23	11	18
Mean (sec)	6.6	8.1	8.4	9.9	24.9
Std dev (sec)	2.6	1.4	1.6	1.9	7.0

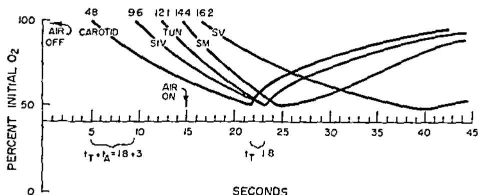


Fig 3. Idealized oxygen-depletion and recovery curves taken from graphic recordings and adjusted for differences in electrode sensitivities When the electrode is close to the oxygen source (stria vascularis and tunnel of Corti), the curves show a relatively sharp reversal point and a variable rate of recovery t_T blood transit time, t_A autoregulation time

During the 'off' period, the oxygen of the tunnel fluid takes 2.5 sec longer to show a decline than does the oxygen of the capillaries of the stria vascularis This is probably related to the very high metabolic rate (Chou & Rogers, 1962) of this tissue

For lack of a better explanation it is assumed *that the longer delays for oxygen depletion and* recovery in the fluids of the scala media and scala vestibuli are the result of a combination of diffusion times and lack of active-metabolic tissue to make use of the oxygen

A distinctive characteristic of the oxygen epletion and restoration curves is their shape, s shown in Fig 3 Of particular interest is the shape of the recovery curve as the oxygen level returns to normal after restoration of the respiratory air For the electrodes located in direct contact with the blood or in a capillary area, there is a sharp reversal after the air is turned on This is followed by a rapid rise at first, a slight break in the curve and then a more gradual rise back to the normal level Such curves are recorded from the carotid, stria vascularis, and tunnel of Corti

In contrast to the curves of the vascular areas, are the more gradual curves with less distinct reversal points from electrodes in the fluid spaces where oxygen availability is determined by diffusion from the capillary networks Such curves are recorded from the scala media and scala vestibuli

B *Analysis of delay factors*

The delays recorded in the tables and illustrated in Figs 2 and 3 can be caused by several factors (*a*) transit time (t_T), the time it takes the oxygen-depleted or enriched blood to pass from the heart (recorded by the carotid electrode) to the labyrinthine recording electrode, (*b*) diffusion time (t_D), *the time taken for the oxygen* to diffuse from a vessel through extracellular fluid space to the recording electrode, autoregulation time (t_A), the additional delay in oxygen depletion at the recording electrode due to the local maintenance of volume flow in response to arterial blood flow changes

It is obvious that such autoregulation can operate over only a limited range of arterial blood-flow variation Consequently, after respiratory air has been interrupted for a while and then restored, autoregulation is no longer involved, only transit and diffusion factors This allows the calculation of transit time from the oxygen-increase delay times, by taking the difference between the reversal time in the carotid artery and the reversal time in a capillary network such as the stria vascularis This is illustrated in Fig 3 (t_T) and gives a time of 1.8 seconds from carotid (representing arterial oxygen) to either capillary area the stria vascularis, or the spiral capillaries supplying tunnel oxygen

The numbers show (Fig 3) a delay of 4.8 seconds between initial-oxygen depletion in the

carotid and in the stria vascularis If 1 8 sec of this time is due to blood transit the remainder should be due to autoregulation (t_A), assuming that diffusion time with the electrode within the strial-capillary network is negligible

As shown in Fig 3, the lag between incipient oxygen depletion in arterial (carotid) oxygen and stria vascularis oxygen is 4 8 sec Allowing for transit time of 1 8 sec, an autoregulation time of 3 0 sec remains

This figure can now be used to find out how much the arterial oxygen must drop before autoregulation can no longer overcome the change by increase in volume flow The percentage drop in arterial oxygen can be calculated by finding the point on the arterial (carotid) oxygen depletion curve corresponding to the end of the autoregulation period In Fig 3 this point is 3 0 sec beyond the initial oxygen fall in the carotid oxygen curve, a fall of about 15% In a number of animals in which this determination has been made the percentage drop in arterial oxygen over which labyrinthine autoregulation can operate is 6–20%

The method of shutting off the respiratory air in order to produce labyrinthine hypoxia is not a procedure that has much application in real life situations but it has served to quantify the autoregulation times presented above and the data provide a basis for the study of ischemic conditions in the labyrinthine system leading to loss of organ of Corti function (sensorineural deafness) There is a built in mechanism providing a margin of safety in maintaining the homeo static condition of the inner ear over a range of changes in arterial blood flow

C *Diffusion times*

It is apparent from Fig 3 that after shut off of respiratory air, the last areas to show oxygen depletion are the endolymph of the scala media and, later, the *perilymph of the scala vestibuli* The same order of events occurs following restoration of respiratory air, with perilymph oxygen of scala vestibuli taking much longer

The active tissue in the scala media is the stria vascularis Here, oxygen is used primarily for strial function and the oxygen recorded in the endolymph is that which diffuses from the stria Following the drop in oxygen supplied to the capillary network of the stria, the remaining *oxygen is used up rapidly by metabolic processes*

In the case of perilymph of the scala vestibuli the situation is different because there is no active tissue The oxygen measured here has diffused from the vessels in the walls, particularly from the capillaries in the spiral ligament "above' Reissner's membrane Upon interruption of respiratory air, the oxygen slowly diffuses away, as there is no tissue actively using oxygen, and with the restoration of arterial oxygen it takes a long time to start to build up again It would appear that the oxygen that diffuses into the perilymph of the scala vestibuli plays no active part in the normal metabolic or transduction processes of the cochlea, but it is certainly available to the cells of Reissner's membrane

RESUMÉ

La teneur en oxygene de l endolymphe cochleaire du cobaye a ete mesurée avec une microélectrode, qui penétrait la zona pectinata de la membrane basilaire et les cellules de Claudius afin de ne pas gener la circulation du sang a l organe de Corti La teneur en O_2 et l amplitude des potentiels cochleaires ont ete enregistrees pendant une hypoxie provoquee par la diminution de l O_2 dans l air inspiré La persistance d un pO_2 endolymphatique normal après la chute de l O_2 arteriel a indiqué la présence d une réserve d oxygene et d une autoregulation de la circulation cochleaire

ZUSAMMENFASSUNG

Die O_2 Konzentration der Schneckenendolymphe wurde beim Meerschweinchen mit einer Mikroelektrode gemessen *Die Elektrode wurde durch die Zona pectinata der* Basilarmembran und Claudiussche Zellen gefuhrt damit die Blutzufuhr zum Cortischen Organ nicht beeintrachtigt wurde Die O_2 Konzentration und die Amplitude des Schneckenpotentials wurden wahrend einer Hypoxie gemessen, die durch Verminderung des O_2-Gehaltes der Atemluft erzeugt wurde Die Aufrechterhaltung der normalen O_2-Konzentration der Endolymphe nach dem Abfall des arteriellen pO_2 deutete auf eine O_2 Reserve und auf eine Selbstregulierung der Schneckendurchblutung.

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DISCUSSION

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EVOLUTION OF CM, SP AND AP DURING ETACRYNIC ACID INTOXICATION IN THE GUINEA PIG

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Abstract During etacrynic acid intoxication in the guinea pig, patterns of click-evoked compound electrocochleographic responses are similar to those observed in Menière and retro-cochlear human pathologies Using high frequency tone bursts, SP, CM and AP were studied after intracardiac injection of etacrynic acid The evolution of SP precedes that of CM and AP Its change in polarity could be related to that reported for EP by Bosher et al. (1973) While during the recovery period SP overshoots (after 90 min), CM and AP increase more [illegible] latency variations are The early positive peak or negative shift observed in click-evoked human pathological electrocochleography can therefore be identified as SP Thus this response is the indication of the presence of hair cells and of the impairment of the nerve fibre stimulation or response

Some pathological patterns of human electrocochleographic responses (promontory recorded, averaged click-evoked compound cochlear nerve action potentials, AP, with cancellation of cochlear microphonic, CM, by summation of responses to acoustic impulses of alternately opposite phase) present a near to 0 ms latency signal, either positive or negative, preceding an action potential either normal-like or broad (Fig 1) The short latency of this signal has suggested that it was a receptor potential either the summating potential (SP) or some residual cochlear microphonic if this was not symmetrical Such early receptor potentials are encountered in Menière ears (when it is mostly negative) and in retro-cochlear disorders such as neurinoma inside the internal acoustic meatus, vascular disorder, kernicterus (where it presents more often as a positive peak) Using tone bursts of various frequencies and studying derived APs, Eggermont (1976) has shown that in such cases a large summating potential was superimposed on an action potential normal (in Menière ears) or broad (in neurinoma)

In various animal experiments we were earlier able to reproduce the other pathological click-evoked patterns corresponding to outer hair cell or nerve fibre losses limited at the base of the cochlea (high frequency hearing loss—*dissociated responses*) (Aran & Darrouzet, 1975) or spread over the whole length of the basilar membrane (flat audiogram with recruitment—*recruiting type responses*) (Aran & Charlet de Sauvage, 1975) In these cases the pathological patterns indicate that the function of the remaining structures is normal, while in the Menière and retro-cochlear cases, besides a possible similar anatomical deficit, there seems to be a dysfunction of the remaining cochlear structures

On only one occasion did we observe in guinea pigs click-evoked responses strikingly similar to those of this latter human category This was during experiments in awake guinea pigs after intra-cardiac administration of etacrynic acid (Aran, 1976) (Fig 2) The electrocochleographic responses were recorded in guinea pigs with a technique and equipment

This work was supported in part by INSERM grants 74 1 087 6 and 75 1 193 6

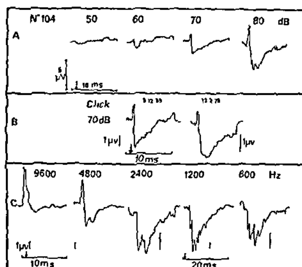


Fig 1 Electrocochleography in a patient presenting with [illegible] responses to the click before and after the surgical removal of the tumour (C) Responses to filtered clicks of various frequencies recorded after surgery Note the positive peaks on the responses to the clicks and high frequency filtered clicks and the negative shifts for the lower frequencies filtered clicks (From Aran, 1973, *Adv Otorhinolaryngol 20*, 389)

identical with those used in earlier experiments (Portmann et al, 1973, Aran & Darrouzet, 1975) and also in human electrocochleography (except for the site of the electrode—round window instead of promontory—and the production and delivery of the acoustic click—0 1 ms rectangular electrical impulse into a TDH 39 Telephonics receiver tube coupled to the ear instead of loudspeaker in free field)

In order to elucidate the nature of the positive peak and of the distorted response pattern produced by the click, we tried to investigate separately but simultaneously the three main components which can show up in the response recorded in such a way the cochlear microphonic, the summating potential, and the nerve action potential

MATERIAL AND METHODS

For that purpose we used tone bursts instead of clicks The sound production system was modified The sound was produced into a polarized Bruel & Kjaer 4134 condenser microphone cartridge reversely used as an ear phone coupled to the ear through a 10 cm Silastic tube (ø 3 mm), sealed within the external acoustic meatus with Histoacryl This acoustic system allows the delivery of sounds with frequencies up to 40 kHz In order to investigate easily the cochlear microphonic and the summating potential, they had to be generated near the recording site, i e near the round window Consequently we used high frequency tone bursts (16 000 Hz), with about 0 3 ms rise time, in order to evoke also a clear AP, and a plateau of usually 5 ms This stimulation was kept constant at an SPL level of 85 dB corresponding to about 70 dB above the AP threshold for this stimulus

The guinea pigs, equipped with an electrode on the round window of the left ear, were tested either awake or asleep, the cartridge being fixed to the socket receiving the electrodes together with the plug connecting these electrodes to the preamplifier, or under light anaesthesia (Ketamine) No differences were noted in the evolution of the responses under these two different conditions

The responses were recorded continuously before and after the intra-cardiac injection of etacrynic acid 200 stimuli of alternately reversed polarity, were presented at a repetition rate of 30/s every 15, 30 seconds, 1, 5, 10, 30 minutes, hours and days, depending upon the speed of the evolution of the responses The responses were amplified with a broad band preamplifier (0 3 Hz–30 kHz) and averaged separately for each polarity of the stimulus in a Histomat S signal analyser system and stored on a digital magnetic tape Adding and subtracting the two averaged signals gave separately the SP+AP and CM alone (Fig 3) Then SP amplitude (from baseline to first positive or negative deflection) AP amplitudes (N1, from top of SP to the trough of N1, occasionally N2) and latencies, and peak-to peak amplitude of CM were measured and plotted as a function of time (injection time = time 0) It must be noted, as pointed out by Gibson & Beagley (1976),

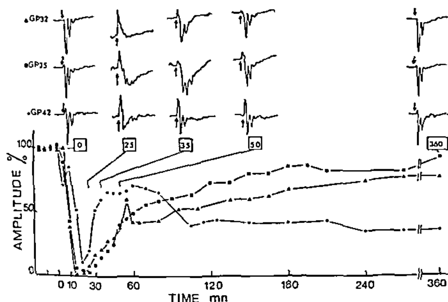


Fig 2 Evolution of the compound electrocochleographic response to a 70 dB HL click in three awake guinea pigs after intracardiac injection of etacrynic acid (30 mg/kg) (time 0) and patterns of the responses at different typical times The amplitudes are expressed as a percentage of their value before the injection, respectively 232, 448 and 576 μV for guinea pigs (GP) 32, 35 and 42. Traces with normalized dimensions Note the positive peaks at 25 and 35 minutes (From Aran, 1976, *Neuroscience Letters 2/6* 335)

that the polarity of SP is here referred to, as in human electrocochleography, as round window (scala tympani) versus ground, which is the reversed polarity of that described by Davis et al (1958) (scala vestibuli versus scala tympani)

Experiments were performed on 9 guinea pigs receiving intracardiac injections of etacrynic acid with doses of 30 mg/kg Two different lots of etacrynic acid (Edecrin) from the same manufacturer but from different suppliers were used, in another guinea pig a dose of 60 mg/kg of one of these two lots was given

RESULTS

The short term transient period of the evolution was studied as well as the long-term modifications

The evolution of the various potentials usually follows the pattern described in Figs 3, 4 and 5 The first signal to be affected is the summating potential, which decreases very quickly Then CM and N1 amplitudes start to decrease too, while the N1 latency increases SP decreases to 0, becomes negative and reaches maximum negativity about 5 to 10 minutes after the injection This time corresponds to the disappearance or minimum amplitude of N1 One should note that although SP changes its polarity, the phase of the reduced CM remains unchanged The SP then returns towards zero while the CM decrease slows down, and reaches a minimum where it stays for a few minutes before starting to recover very slowly as compared with the evolution of SP and AP A few minutes after SP crosses zero and becomes positive, N1 is restored, with a very small amplitude and a long latency While SP, about an hour after the injection, has returned to its initial value and even overshoots, CM and N1 stay at about 50 % of their initial value One must wait between 7 to 10 days to observe a total recovery of CM while, in all the cases but one, N1 never recovered completely and stayed at about 70 to 80 % of its initial value, even 25 days after the injection SP in the long term is much more difficult to characterize, no clear tendency could be observed, as it was found both larger and smaller than before the injection

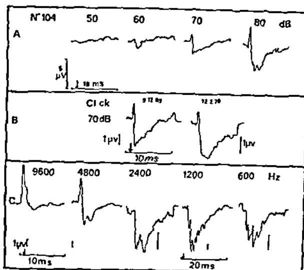


Fig 1 Electrocochleography in a patient presenting with

responses to the click before and after the surgical removal of the tumour (C) Responses to filtered clicks of various frequencies recorded after surgery Note the positive peaks on the responses to the clicks and high frequency filtered clicks and the negative shifts for the lower frequencies filtered clicks (From Aran, 1973, *Adv Otorhinolaryngol 20* 389)

identical with those used in earlier experiments (Portmann et al, 1973, Aran & Darrouzet, 1975) and also in human electrocochleography (except for the site of the electrode—round window instead of promontory—and the production and delivery of the acoustic click—0 1 ms rectangular electrical impulse into a TDH 39 Telephonics receiver tube-coupled to the ear instead of loudspeaker in free field)

In order to elucidate the nature of the positive peak and of the distorted response pattern produced by the click, we tried to investigate separately but simultaneously the three main components which can show up in the response recorded in such a way the cochlear microphonic, the summating potential, and the nerve action potential

MATERIAL AND METHODS

For that purpose we used tone bursts instead of clicks The sound production system was modified The sound was produced into a polarized Bruel & Kjaer 4134 condenser m phone cartridge reversely used as an ear ph coupled to the ear through a 10 cm Sil tube (⌀3 mm), sealed within the ext acoustic meatus with Histoacryl This acou system allows the delivery of sounds with quencies up to 40 kHz In order to investi easily the cochlear microphonic and the s mating potential, they had to be generated n the recording site, i e near the round wind Consequently we used high frequency tone bu (16 000 Hz), with about 0 3 ms rise time, order to evoke also a clear AP, and a plat of usually 5 ms This stimulation was kept c stant at an SPL level of 85 dB correspond to about 70 dB above the AP threshold for t stimulus

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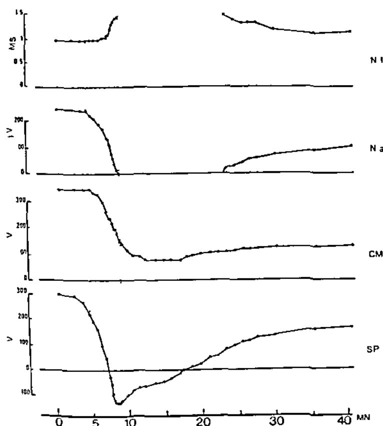


Fig. 4. Short term evolution of N1 latency (*N1l*) and amplitude (*N1a*) and of CM and SP amplitudes before and after the intracardiac injection of etacrynic acid (time 0) (30 mg/kg). Same guinea pig as in Fig. 3. The two dotted lines indicate the time N1 starts to decrease and the time it disappears.

(amplitudes and slopes of polarity changes). However, this does not clarify the origin of SP which is indeed produced inside the cochlear duct and depends upon the hair cells function and the d.c. gradient. It is obvious that only the simultaneous recording of EP with all the other potentials will elucidate the relationships between hair cells, endocochlear potential, and nerve fibre excitation.

The increase in latency of N1 also indicates

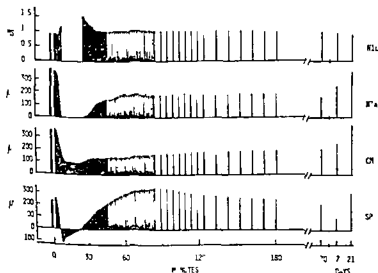


Fig. 5. Evolution of N1 latency (*N1L*) and amplitude (*N1A*) and of CM and SP amplitudes in another guinea pig during minutes and at 7 and 21 days (right of the dotted line) after the intracardiac injection of etacrynic acid (30 mg/kg) (time 0).

the progressive impairment of the basal turn of the cochlea The 85 dB SPL 16 kHz burst stimulates from the oval window to the 16 kHz area on the basilar membrane (about 2 mm) Thus the latency is brief When the hair cell function is severely impaired, only the area corresponding to the maximum mechanical sensitivity is initiating nerve responses, with a time delay thus corresponding to the travelling time from the oval window to this area In effect this longest latency has the same value as that obtained in a normal cochlea with an identical very low intensity stimulus (threshold)

CONCLUSIONS

On reconsidering the similarity between click evoked responses in human Meniere and retro cochlear pathologies to those occasioned in guinea pigs by etacrynic acid intoxication, and taking into account the observations of Eggermont on SP in patients, it can be safely concluded that the early positive peak, or negative shift, in the response to the broad frequency spectrum click is a summating potential which of course with the transient character of this stimulus, is very brief

When such a response is observed it can be inferred that there is a disturbance of the cochlear fluids and/or of the hair cells The presence of the large summating potential with respect to the sometimes small AP, guarantees the existence of hair cell activity but demonstrates that the nerve fibres are not effectively triggered, whether this is due to impairment of hair cell function and/or of the nerve fibres

ACKNOWLEDGEMENT

The authors wish to thank Mr J P Erre for the preparation of the animals and his help during the experiments and Mr Y Cazals for his participation in the preparation of the manuscript

RESUMÉ

Au cours de l intoxication par l acide étacrynique chez le cobaye, les formes des réponses électrocochleographiques au clic sont semblables à celles observées chez l homme dans la maladie de Menière et dans les troubles rétro-cochléaires Le potentiel de sommation (PS), le potentiel microphonique cochléaire (MC) et le potentiel d action (PA) ont été étudies en utilisant des sons brefs de haute fréquence chez le cobaye après injection intra cardiaque d acide étacrynique L evolution du PS précède celle du MC et du PA Son inversion de polarité peut etre comparée à celle observée sur le potentiel endocochléaire (PEC) par Bosher et coll (1973) Tandis que pendant la période de recupération le PS dépasse son amplitude initiale (apres 90 min) le MC et le PA augmentent plus lentement (pendant près de 7 jours) et dans la plupart des cas l amplitude du PA n atteint que 70 à 80 % de sa valeur initiale (25 jours) Quand N1 est présent il garde toujours une forme normale quelque soit son amplitude et sa latence Le pic positif précoce ou la déflection negative rencontres dans les réponses électrocochléographiques au clic dans certaines pathologies humaines peuvent être identifiés comme PS Cette reponse indique donc la présence de cellules ciliées mais un défaut dans la stimulation ou dans la réponse des fibres nerveuses

ZUSAMMENFASSUNG

Die elektro cochleographischen Reaktionen des Meerschweins auf das Klick nach einer intracardiac Injektion von Ethacrynsäure (30 mg/kg) gleichen den anormalen Reaktionen beim Menschen im Falle von Ménere vaskularen und retro-cohlearen Storungen D e detail

wird prasentiert

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DISCUSSION

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OLFAKTORISCHE STÖRUNGEN BEIM HYPOGONADISMUS

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Abstrakt Auf Grund des Studiums und im besondern [illegible]

Storungen bestehen Dieses Syndrom, beschrieben als Kallmanns oder De Morsiers Krankheit, ist wenig bekannt, verdient aber unsere Beachtung weil es auf Läsionen im Bereich zwischen Hypothalamus und der Hypophyse hinweist

Die Bedeutung und die gegenseitige Beziehung des Geruches und der Sexualsphäre sind nicht unbekannt und sie waren der Gegenstand experimenteller und klinischer Forschungen Der Einfluß des Geruches auf die Pathophysiologie der männlichen und der weiblichen Geschlechtsorgane und umgekehrt ist ein interessantes Kapitel der klinischen Olfaktologie und der Endokrinologie

Fur die ausgeprägte Wirkung des Geruches auf die Tatigkeit der Geschlechtsdrusen sind in der Zoologie viele Beispiele vorhanden Sie wurden von zahlreichen Autoren beschrieben Roeder, Kitajima, Le Mangen, David, Y Guerrier und R Azemar, deren Forschungsergebnisse unbestreitbare Beweise fur die wechselseitige Wirkung des Geruchssinnes und der Geschlechtsfunktion bieten Sie weisen auf den Einfluß olfaktorischer Empfindungen auf den hypophysär-genitalen Mechanismus hin, der gegenseitig ist

Demgegenuber sind solche Beobachtungen an Menschen sehr spärlich, genugen aber fur den Hinweis auf die Tatsache, daß die Geschlechtsdrusen durch ihre innere Sekretion die normale anatomische Entwicklung des Riechapparates und sein physiologisches Funktionieren ermöglichen

Unsere eigenen Untersuchungen mögen auf die Folgen jener pathologischen Zustände hinweisen, die auf den Zusammenhang zwischen dem Geruch und dem Geschlechtssinn beruhen Es werden Fälle mit olfaktorischen Störungen bei Hypogonadismus die als Kallmannsches (Labhart, 1971) oder als De Morsiersches Syndrom (de Morsier & Gauthier, 1963) beschrieben wurden, beschrieben

Das Kallmannsche Syndrom, oder, nach franzosichen Autoren, das De Morsiersche Syndrom, ist eigentlich eine olfaktogenitale Dysplasie, die durch eine vollständige oder partielle, beider oder einseitige Agenesie des Geruchslappens des Großhirns mit Mangel an gonadotropen Hormonen gekennzeichnet ist. Das fuhrt zum klinischen Bild des sekundären Hypogonadismus Der primäre Hypogonadismus geht dagegen mit erhöhten Gonadotropinwerten einher, während der tertiäre die Folge verschiedener Endokrinopathien oder anderer Erkrankungen ist Das Kallmannsche Syndrom ist das Ergebnis einer gleichzeitigen Hypothalamusschadigung oder sogar der suprahypothalamischer Zonen, wobei die Hypophyse selbst nicht befallen ist Es manifestiert sich mit zwei Symptomengruppen

innersekretorisch, d h Hypogonadismus der sich erst zu der Pubertätszeit außert Dieser Hypogonadismus ist isoliert und ist nicht mit anderen Funktionsstörungen der Adenohypophyse begleitet Das Hormonniveau zeigt sehr niedrige oder unmeßbare Gonadotropinwerte Der Wuchs des Patienten ist meistens normal, jedoch mit eunuchoidem Körperbau.

neurologische, welche als Storung der Geruchsfunktion (von totaler Anosmie bis an die partielle Schädigung bezüglich einzelner Geruche) beschrieben wurden Diese Geruchsstorungen sind nicht auf anatomische Veränderungen in der Nase zuruckzuführen Außer den olfaktorischen können auch andere neurologische Ausfalle vorhanden sein

Die Krankheit kommt häufiger bei Männern vor Die Frage ihrer Vererbung wird noch diskutiert Bei Familienangehörigen solcher Patienten sind rudimentare Formen olfaktogenitaler Dysplasie beobachtet worden Man nimmt an, daß die Krankheit dominant vererbbar ist

Bei der Diagnosenstellung des männlichen Hypogonadismus halten wir uns hauptsächlich an das Diagnosenschema aus dem Buche Labharts, welches unseren diagnostichen Moglichkeiten angepaßt wurde

In den Jahren 1974 und 1975 hatten wir die Gelegenheit, 5 Fälle solchen Syndroms zu beobachten Bei allen Fällen weisen die klinischen und laboratorischen Befunde unbestreitbar auf die Diagnose des sekundären Hypogonadismus hin Für uns sind dabei die olfaktometrischen Befunde interessant

Die Untersuchung der Geruchsfunktion bei unseren Kranken zeigten, daß 3 Falle davon total anosmisch waren, während die übrigen 2 eine gestörte Identifizierung der Geruche aufwiesen, was mit den fruheren Ergebnissen mancher Autoren in Einklang steht Bricaire, Franchimont und Luton haben nämlich in ihrer Serie von 4 Patienten nur eine Anosmie und 3 Fälle von Hyposmie gehabt (Bricaire et al, 1972)

Bei der Besprechung unserer Ergebnisse wurden wir feststellen, daß sie im Einklang mit den Voraussetzungen fruherer experimentellen Arbeiten steht, die auf die gegenseitige Beziehung der Geruchsbahnen und der Geschlechtssphare hingewiesen haben Die olfaktorischen Reize gelangen, auf noch bisher unbekannten Wegen, bis in das Rhinencephalon (nicht nur in das primäre Riechzentrum) Dieser ist mit dem hypothalamisch-hypophysären Bereich des Gehirns, wovon die Geschlechtsfunktionen reguliert werden, verbunden Die afferenten Geruchsempfindungen werden sehr gestreut, so daß der ganze Rhinencephalon auf olfaktorische Reize reagiert Das ganze limbische System ist sogar einbegriffen Ruckwirkend wird die Empfindlichkeit des peripheren Riechsystems durch den Rhinencephalon und die subkortikalen Zonen beeinflußt So ist die periphere Geruchsempfindlichkeit der zentralen Steuerung unterworfen Das heißt, daß der Geruch bei Normalen immer die periphere und die zentrale Stimulation hervorrufen wird Anderseits ist das Rhinencephalon die Stelle des Integrierens und der Regelung des Geschlechtsverhaltens (was beweisbar ist durch die Änderung elektrischer Tätigkeit des Riechhirns der Hasin während der Paarung) Die Schädigungen dieser gegenseitig verbundenen Strukturen können erworben oder geerbt sein, was der Fall bei unseren Patienten war

SCHLUSSFOLGERUNG

1 Vom theoretischen Standpunkt ist dieses Syndrom interessant, weil es nochmals auf die Verbundenheit des zentralen Nervensystems mit dem innersekretorischen Apparat hinweist

2 Dieses Syndrom, beschrieben als Kallmanns oder De Morsiers Krankheitsbild, ist wenig bekannt, verdient aber unsere Beachtung, weil es auf Läsionen im Bereiche zwischen dem Hypothalamus und der Hypophyse hinweist

3 Gleichzeitig wiesen diese Untersuchungen auf neue diagnostische und wahrscheinlich auch therapeutischen Möglichkeiten hin

4 Systematische olfaktometrische Untersuchungen und das Diagnostizieren der Riechfunktionsstörungen (welche der Patient sonst selten spontan anzugeben pflegt) wird zu häufigeren Entdecken solcher Fälle beitragen

5 Unser Ziel war es, auf ein Syndrom hinzuweisen, das häufiger ist als man es früher angenommen und diagnostiziert hat

SUMMARY

On the basis of the study and particularly of the examination of the olfactive functions in three patients with hypogonadismus the relationship between the olfactive and the endocrine lesions is established This syndrome, described by Kalmann and by De Morsier is not well known, but it deserves attention since it is indicative of pathologic changes in the region which lies between the hypothalamus and the hypophysis

RÉSUMÉ

Sur la base des études et particulièrement des examens d'olfaction chez trois malades montrant l'hypogonadisme, on etablit les relations existant parmi les troubles olfactifs et endocrines Ce syndrome decrit sous le nom de Kalmann ou bien de Morsier est peu connu, mais il mérite toute attention parcequ'il témoigne des lésions situées entre l hypothalamus et l'hypophyse

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DISCUSSION

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INTRATHORACIC INJURY TO THE MOTOR NERVE SUPPLY OF THE LARYNX

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Abstract A previously reported experimental study demonstrated that the intermediate (partly abducted) position of a paralysed vocal cord may be due to physiological inactivation rather than paralysis, of the cricothyroid muscle This inhibition was shown to be caused by interruption of vagal afferent impulses originating in pulmonary pressure receptors The case reported here offered an unusual opportunity of studying this condition by serial section of a human larynx A patient's left vocal cord was paralysed by cancer in the left pulmonary hilum and apex. There was aspiration and loss of voice The larynx was obtained at autopsy and studied by serial sections These showed that the P C A muscle on the paralysed side was, in fact, completely atrophic and degenerated, as were the other intrinsic muscles, but that the cricothyroid muscle was morphologically normal Invasion of both the recurrent laryngeal nerve and the vagus at the thoracic inlet interrupts afferent impulses and inactivates the cricothyroid muscle, with resultant glottic incompetence

The motor nerve supply to the larynx may be damaged at the intrathoracic level by disease or trauma In this circumstance, does the paralysed vocal lie in a paramedian or an intermediate (partly abducted) position? Both positions have been reported, not only in association with acute but also with slowly progressing damage to the motor nerve supply within the chest

Baranyai & Madarasz (1963), for example, reported 3 cases of vocal cord paralysis following lung operations In all 3 patients, the paralysed vocal cord lay in the paramedian position The authors' remark that "since in lung cases only the recurrent laryngeal nerve can be affected, it is quite clear why we could find the cords in paramedian position only"

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On the other hand, Lewy & Mathews (1965) reported 3 cases of vocal cord paralysis immediately following intrathoracic surgery, with "the classical clinical picture of a lateral-lying vocal cord and glottic valve insufficiency" One of these patients had a partial aortectomy, the other 2 had pneumonectomy for malignant growths In one pneumonectomy the resection included the recurrent laryngeal nerve

In my own experience, glottic incompetence has been observed in 3 patients during the immediate postoperative period following cardiovascular procedures The vocal cord lay slightly lateral to the paramedian position in 2 patients and in the intermediate position in the third All regained complete function in 3 months or less

In a slowly developing paralysis, the glottic picture is still more difficult to explain Semon (1881) distinguished between "an acute and a gradually progressive lesion of the nerve" and held the view that in the latter case, the abductor fibers of the RLN lose their conductivity sooner than the adductor fibers and that the paralysed vocal cord assumes and maintains the paramedian position because of the continuing activity of the adductor muscles However, his reports make no mention of a vocal cord, initially paralysed under these conditions, eventually moving to a more lateral position as the adductor muscles become paralysed

Mackenzie (1880) also held the view that the abductor muscle fibers were more vulnerable to neurogenic injury than the adductors, but added

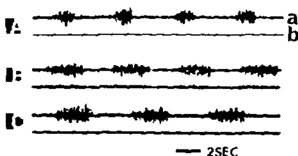


Fig 1 Cricothyroid muscle inhibition by intrathoracic interruption of the vagus nerve in the presence of recurrent laryngeal nerve paralysis (cat) (*a*) EMG of diaphragm in all three tracings (*b*) EMG of cricothyroid muscle in all three tracings (*A*) Moderately deep anesthesia. No activity of cricothyroid muscle (*B*) Lighter anesthesia, left recurrent laryngeal nerve cut Motor unit in cricothyroid muscle becomes active (*C*) Continuation of (*B*) but vagus nerve now compressed with forceps at level of clavicle Cricothyroid activity is abolished

the hypothetical factor of partial injury to the nerve The condition of the larynx "depends on whether the paralysis is complete, involving the whole trunk, or whether it is partial, involving the filaments going to the abductor alone"

The adductor power of the cricothyroid muscle was generally overlooked in the late 19th century reports Mackenzie's textbooks, for example, describes complete paralysis of the RLN as producing a cord paralysed in the "cadaveric sition"

It is difficult to draw conclusions from published reports because most of them lack some essential point of information In many reports, the position of the paralysed vocal cord is described, but no mention made of vocal quality, stridor or air loss Even reports of post-mortem examinations often fail to state whether the "normal" adductor muscles were so identified by mere inspection or whether they were examined microscopically More important, the conditions of the cricothyroid muscle is rarely mentioned

Other 19th century and more recent reports attribute the position of the paralysed vocal cord to forces other than abductor and adductor muscle activity or its absence

Luschka (cit by Semon) observed that vocal cord movements "are not exclusively results of the direct action of the muscles Dilatation and constriction of the different laryngeal compartments can, up to a certain degree, be produced as well by the elastic membrane of the larynx returning to its former state, after certain muscles, which brought it into a state of tension, have ceased to act This state of things much resembles that acting upon the thorax during inspiration and expiration"

Kanthack (1892) prepared celloidin sections of the larynx and attributed part of the glottic picture to the preponderance of sphincteric over dilator muscle fibers

Grossman (1906) stated that "after a period of paralysis of the recurrent laryngeal nerve, the cricothyroid muscles undergo 'disuse atrophy' because they no longer have to pull against the antagonistic thyroarytenoid muscles which have been paralyzed" This is probably the first reference to a physiological inactivation of the cricothyroid muscle as contrasted with actual paralysis

LeMere (1932) used Grossman's experimental evidence to explain the occasional case in which paralysis of the RLN is lessened with the passage of time, i e the cricothyroid's atrophy and a more intermediate position of the cords is substituted for a partially adducted position

Oltersdorf (1952) reported that vocal cord position depends largely on the mechanics of the arytenoid cartilage The lever arm of the posticus is small The lever arm of the adductors is large because it inserts farther from the joint surface He also pointed out that the bulk of the adductor muscles is greater than that of the abductor

Moser (1956) postulated that neurodegenerative changes vary in degree and often affect only some of the nerve fibers

Fink et al (1956) called attention to the effect of passive forces within the larynx including those produced by the external muscles

Kecht (1956) reported that topical or local anesthesia applied to the larynx produced a widening of the glottis

Zenker & Zenker (1960) called attention to



Fig 2 Coronal section of anterior larynx viewed from front Upper arrow Normal right thyro-arytenoid muscle On the opposite side the thyro-arytenoid muscle is atrophic and largely replaced by fibrous tissue Lower arrow Normal right cricothyroid muscle On the opposite side this same muscle is normal by gross and microscopic examination

the effect of varying amounts of fat and connective tissue in and around the larynx

Faaborg Andersen (1964) revised the idea of partial paralysis and showed that immobile vocal cords often demonstrate some residual electromyographic activity

Regardless of terminology a paralysed vocal cord lies either at the midline or near enough to it for normal phonation—or it lies far enough lateral to the midline to allow air loss and hoarseness The glottis in this latter condition can be said to be incompetent

A lateralized vocal cord is seen in its most extreme form in injuries to the vagus nerve at the ganglion nodosum, or near enough to it to involve the superior laryngeal nerve trunk As a result, all the laryngeal muscles including the cricothyroid are paralysed, except for the bilaterally innervated inter arytenoid and the vocal cord assumes the intermediate position Aphonia and breathlessness result (Kirchner, 1966)

However, the incompetent glottis can also be the result of injury to the motor nerve s

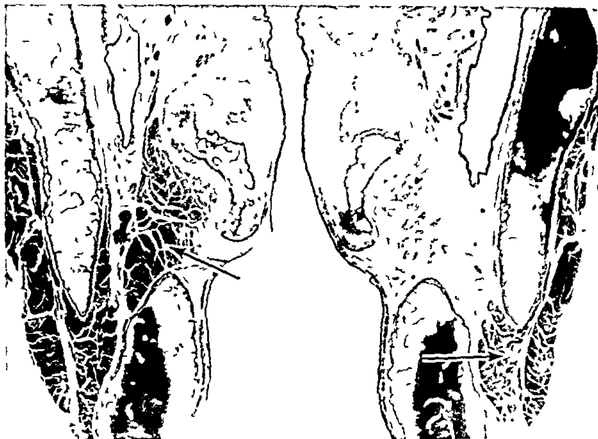


Fig 3 Coronal section further posteriorly Upper arrow indicates right lateral cricoarytenoid muscle On the opposite side this muscle and the external thyroarytenoid muscle immediately above it are atrophic and fibrotic The cricothyroid muscle (lower arrow) on the paralysed side appears normal

mediastinum Aortic aneurysm, apical tuberculosis, cancer of the upper lobe or hilum and similar conditions often produce a vocal cord paralysed in a partly lateralized position, with partial or complete aphonia and air loss

Why should an intrathoracic lesion produce a midline paralysis of the vocal cord in some cases and a partly lateralized position in others?

The concept of partial injury to the recurrent laryngeal nerve was dealt a severe blow by the work of Sunderland & Swaney (1952), who showed that the adductor and abductor fibers in the RLN are thoroughly intermixed along their entire extra laryngeal course Certainly at the intrathoracic level it would be impossible for a partial injury to the nerve trunk to produce selective damage to the abductor or adductor fibers

Another explanation of glottic incompetence in case of intrathoracic disease was offered by Hofer (1953), who reported a cadaveric position in each of 4 patients, 2 with bronchogenic carcinoma, one with carcinoma of the esophagus and one with aortic aneurysm In these cases the left vagus nerve was completely surrounded by cancer or, in the aneurysm splintered out into fine branches Hofer observed that a paralysed vocal cord assumes the intermediate position if the vagus nerve is interrupted When only the RLN is interrupted, however, the cord position is paramedian He proposed the explanation that tigrolytic degeneration of the ganglion cells in the nucleus ambiguus results from vagal interruption in the mediastinum and that this might lead to paralysis of the cricothyroid muscle

Although his first observation may be accurate one major objection to his explanation is that the intermediate position of the paralysed cord may be observed almost immediately after

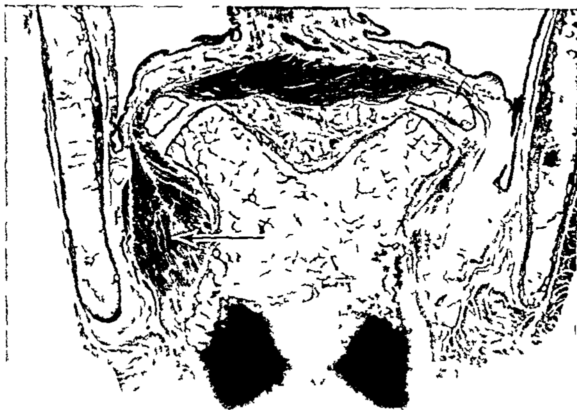


Fig 4 Coronal section posterior larynx Arrow indicates the normal right posterior cricoarytenoid muscle Its fellow of the opposite side is atrophic and degenerated

injury to the motor nerve supply within the chest as after a cardiothoracic operation These patients are sometimes seen in the recovery room completely aphonic and unable to close the glottis The recovery of vocal cord function within a few weeks or months in many of these patients also suggests a cause that is mechanical or reflex rather than degenerative

The mechanical or reflex concept gained further support from the observations of Suzuki et al (1970) who demonstrated that the motor activity of the CT muscle changes quickly in response to variations in intrathoracic pressure Additional support for the concept of reflex inhibition of CT activity was provided by the work of Fukuda & Kirchner (1972) who demonstrated that in the presence of a unilateral paralysis of the RLN intrathoracic interruption of the vagus nerve inactivates the CT muscle and allows the vocal cord to assume a more lateral position

Glottic incompetence in laryngeal paralysis has not yet been fully explained but the answer may gradually emerge through reports of carefully documented cases Studies must include detailed histories precise observation of vocal quality and vocal cord position EMG of the various laryngeal muscles and wherever possible whole-organ sections of the post mortem larynx The present report does not provide this spectrum of information but furnishes evidence that an incompetent glottis may exist in the presence of a morphologically normal cricothyroid muscle

CASE REPORT

A 65-year-old white man with a history of tobacco and alcohol abuse suddenly became hoarse Over the next 3 months he lost 20 pounds in weight

Examination revealed a breathless, whispering voice and a left vocal cord paralysed in the "paramedian" position X-ray of the chest revealed a mass in the left hilum A pulmonary angiogram revealed a circumferential narrowing of the left pulmonary artery Scalene node biopsy showed a focus of metastatic adenocarcinoma Laminograms and cine studies of the larynx showed a "flaccid" paralysis of the left vocal cord

Radiotherapy was delivered to the left hilum, mediastinum and supraclavicular area There was no significant change in the X-ray appearance of the lesion at the end of treatment He was started on cytosine arabinoside, but continued to fail steadily over the next 3 weeks and died During this final admission his voice was very weak, with loss of air

Autopsy showed severe scarring and residual adenocarcinoma of the left upper lobe Left hilar nodes were filled with tumor

DISCUSSION

e intrinsic laryngeal muscles in this larynx re all atrophic and degenerated, just as one would find in a long-standing paralysis resulting from injury to the RLN in the neck In this latter condition, however, the voice is usually normal or serviceable, with no significant air loss This is generally attributed to the adductor and tensor activities of the cricothyroid muscle which holds the vocal cord in or near the midline for the duration of the paralysis Faaborg-Andersen (1964), in a study of 194 patients with vocal cord paralysis following thyroidectomy, reported not a single case in which a cord originally paralysed in the paramedian position moved to an intermediate position

By contrast with the usual long standing paralysis of the RLN, this patient's glottis was incompetent The voice was weak, hardly more than a whisper The cord was reported as occupying a "paramedian" position Regardless of terminology, the aphonia indicates a partly open glottis and a weakness of cricothyroid muscle function in addition to paralysis of the internal laryngeal muscles Despite the duration of the paralysis (7 months at least) there is neither evidence of 'disuse atrophy' nor disintegration of muscle fibers resulting from degenerative changes in the central motor nucleus

Microscopic examination showed the cricothyroid to be morphologically normal

Two explanations of the glottic picture might be considered

(1) The paralysed vocal cord may initially have assumed a position at the midline or near enough to allow normal phonation, because of the adductor power of the cricothyroid muscle As the adductor muscle became progressively more atrophic and fibrotic, the vocal cord could no longer be brought into a position allowing phonation, despite the normal action of the cricothyroid muscle, Or,

(2) The normal function of the cricothyroid muscle was inhibited by the interruption of afferent vagal stimuli resulting from invasion of the vagus nerve by cancer in the left upper lobe Without the normal tensor effect of the cricothyroid, the atrophic vocal cord could not be brought and held close enough to the midline to allow phonation

The one diagnostic test that might have clarified the mechanism of aphonia in this patient is electromyography of the cricothyroid muscles Unfortunately, this was never obtained

RÉSUMÉ

. . . n ibl ée a démontré que . . . vocale paralysée . . ôt que paralysie, . ibition est le résultat d interruption des impulsions afférentes du nerf pneumogastrique, prenant ses origines dans les récepteurs de pression du poumon Le cas qui est présenté . . . extraordinaire de vérifier . . a corde . e cancer . . l aspira tion et la perte de la voix Le larynx procuré à l autopsie

est etudié par les sections en serie Ceux-ci ont demontré que le muscle crico-arytenoidien posterieur sur le coté paralysé était, en fait, tout à fait atrophique et dégeneré, ainsi que les autres muscles intrinsèques, mais que le muscle crico-thyroidien etait normal, au point de vue de morphologie Ce cas confirme l observation experimentale qu'une paralysie d une corde vocale dans la position intermediaire peut être le résultat d inactivation physiologique plutot que paralysie ou atrophie du muscle crico-thyroidien

ZUSAMMENFASSUNG

Eine kurzlich veroffentlichte tierexperimentelle Untersuchung zeigte, daß die intermediare (teilweise abduzierte) Stellung eines paralysierten Stimmbandes eher physiologischer Inaktivierung als der Paralyse des M Cricothyroideus zugeschrieben werden kann Zusatzlich kann diese Stellung der Stimmritze trotz kompletter Lahmung des *M Cricoarytenoideus posticus bestehen* In dem vorliegenden Fall war das linke Stimmband des Patienten von einem Carcinom der linken Lungenspitze und Hilus gelahmt Der Stimmritzenverschluß war unvollständ g und es bestand Aspiration und Stimmverlust Der Kehlkopf wurde bei der Sektion herausgenommen und mit Serienschnitten untersucht Diese zeigten, daß der *M Cricoarytenoideus posticus auf der paralysierten* Seite tatsächlich komplett atrophiert und degeneriert war ebenso wie die anderen inneren Kehlkopfmuskeln, daß aber der M Cricothyroideus morphologisch normal war Auf Grund der Studie scheint mechanische Unterbrechung der afferenten Vagusfasern Ursache dieser Inaktivierung zu sein

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DISCUSSION

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ORGAN CULTURE OF THE AVIAN AND MAMMALIAN OTOCYST

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Abstract A chemically defined medium supplemented with serum has proved suitable for the growth of the isolated embryonic otocyst of both avian and mammalian provenience The results lend further support to the value of the technique and confirm the findings of previous authors [A film was presented]

The relative inaccessibility of the inner ear, protected by the massive bony structures of the temporal bone, has stimulated the development of alternative experimental methods It is interesting to point out that the otocyst was the first embryonic organ rudiment to be grown *in vitro*

Fell (1928–29) successfully cultured the excised fowl embryo otocyst and subsequently Friedmann (1956) has described the full differentiation the sensory areas of the chick embryo inner ar grown in tissue culture using a modified watch glass technique described by Fell & Robison in 1929 (Friedmann, 1959, Friedmann & Bird, 1961 *a*, *b*, Friedmann, 1967, 1969, 1974)

The dissociation and reaggregation of the chick embryo otocyst was investigated by Orr (1968) Orr has also studied (1975) the effect of trypsin on the developing otocysts, causing changes in the basal lamina as described by us in cultures of embryonic salivary gland rudiments (Friedmann & Hodges, 1975)

The mammalian ear has proved more difficult to grow Lawrence & Merchant (1953) cultured the 9 day-old rat embryo otocyst on plasma clots and emphasized the importance of disturbing the growing cultures as little as possible during incubation Van de Water & Ruben have been the pioneers in this field

These authors have perfected the method of organ culture and have achieved complete differentiation of the isolated mouse embryo otocyst (12 day gestation period) of the normal mouse (Van de Water & Ruben, 1971, 1973, 1974) Organ cultures of explanted homozygotic and heterozygotic Kreisler mouse embryo otocysts displayed aberrant development of the cartilaginous capsule (Van de Water & Ruben, 1974)

MATERIAL AND METHODS

Explants

In the present study both chick embryo otocysts and mouse embryo otocysts have been cultured Three-and a half day old chick embryo otocysts used Pregnant mice of the C57 ICRF strain were killed by neck dislocation under light CO_2 anaesthesia 12 days after detection of vaginal plugs The foetuses were removed, decapitated and the otocysts dissected with some of the surrounding mesenchymatous tissues

Fig 1 Low power view of two joint chick embryo otocysts showing the developed otocyst after 12 days *in vitro* ×40

Fig 2 Detail of Fig 1 showing the basal papilla (" organ of Corti) covered by a tectorial membrane-like PAS-positive layer

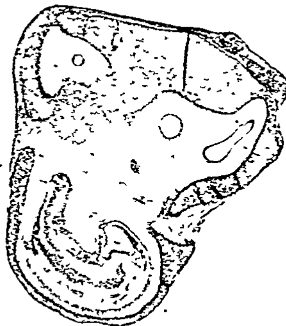
Fig 3 Frame of time lapse film showing the cultured mouse embryo otocyst under phase contrast The curved spiral cochlear duct is visible (*bottom*) and also the semicircular canals (*left* and *top*) ×40

Fig 4 Photomicrograph of Fig. 3 sectioned and stained with PAS Note cochlear duct with basal papilla (*bottom*) and transected semicircular canals (*top*) also well developed cartilaginous otic capsule with gaps for entry of nerves ×60


1


2


3


4

Culture technique

The otocysts were grown on cellophane strips (previously sterilized in 70% alcohol, washed twice in Hanks' saline solution over a period of 60 min and equilibrated in culture medium), then laid over a 3-4 mm hole punched in a stainless steel wire mesh square lying flat on the bottom of optical Anumbra glass Petri dishes The amount of medium added (1 3 ml) came to the level of the cellophane strip

The cultures were incubated at 37°C in sealed humidified Perspex chambers under an atmosphere of 5% CO_2 in air

Culture medium

The culture medium consisted of antibiotic free Waymouth's MB 752 medium (Waymouth, 1959), supplemented with 0 45 μg/ml ferrous sulphate and with 5% calf serum (Flow Laboratories)

Microscopy and filming

Living cultures were photographed and filmed by a time lapse camera technique for 14 days

RESULTS

The sensory areas of the chick and mouse embryo otocyst showed complete differentiation both at light and electronmicroscope levels [as was illustrated by a film presented at the meeting]

Figs 1 and 2 show the chick embryo otocyst and the developed cochlear area The sensory area of the basal papilla with a PAS positive quasi tectorial membrane is shown The cartilage is well developed and there is on the surface an epidermoid structure resembling a small epidermoid cyst (cholesteatoma")

Fig 5 Ciliated surface of hair cell with kinocilium basal body and stereocilia containing microtubules or microfilaments Chick embryo otocyst 12 days *in vitro* 27 500

Fig 6 Neurones of spiral ganglion and multiple nerve axons As Fig 5 × 12 000

Figs 3 and 4 show the fully grown mouse embryo otocyst with particular reference to the spiral developing cochlea and organ of Corti

Fig 5 illustrates the ciliated surface of a hair cell of the vestibular apparatus

Fig 6 shows well developed neurones of the spiral ganglion in the cultured chick embryo otocysts

[NB Additional illustrations were presented with the film and are to form the basis of a more extensive paper]

DISCUSSION

Organogenesis is a complex multi step process and tissue culture methods are invaluable for the study of biological processes at the cellular level The use of a chemically defined medium supplemented with serum has proved most suitable for the organ culture and growth of the developing embryonic otocyst avian and mammal This confirms our earlier studies and lends support to the pioneering work of Van de Water & Ruben on organ cultures of the mouse embryo otocyst The sensory areas of the inner ear, the auditory nerve and neurones reach full development and high degree of ultrastructural differentiation, although separated from the developing central nervous system The method is eminently suitable for the study of ototoxicity, toxoplasmosis, viruses, developmental anomalies and fundamental histological structures such as innervation

ACKNOWLEDGEMENTS

Our thanks are due to Dr L. M Franks for his encouraging interest and to Dr R J Ruben for valuable discussions during his visit to our department

RÉSUMÉ

Une croissance réglée et ordonnée est essentielle pour le développement normal des tissues et la culture organotypique est une méthode de choix pour l étude in vitro de la cytologie de l oreille interne en voie de différenciation L'utilisation d un milieu synthétique additionné de sérum c est montré tres favorable à la croissance de

l otocyste d embryon de mammifere in vitro [La différenciation de l organe est décrite et a été illustrée par un film]

ZUSAMMENFASSUNG

Ein synthetischer Nahrboden mit Serumzulage hat sich sehr gut zur Zuchtung des embryonalen Innenohres der Maus bewiesen Diese Methode unterstuzt das geregelte Wachstum des Ohrkomplexes und eignet sich sehr gut zum Studium der normalen und abnormalen Entwicklung des Ohres wie andere Autoren es bewiesen haben

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DISCUSSION

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INNER EAR MORPHOLOGY IN DOWN'S SYNDROME

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Abstract A comparative study was made on four pairs of temporal bones from patients with Down's syndrome (trisomy 21) and 15 pairs of temporal bones from other infants of the same age range Spiral reconstructions showed cochlear length to be slightly shorter in temporal bones from patients with Down's syndrome than that in the controls Based upon these dimensional measurements, a developmental anomaly of the vestibular apparatus was found

Although much information has been published on temporal bone findings in association with trisomy 13-15 and 18, reports of such findings in association with trisomy 21 are meager No description of temporal bone characteristics in patients having Down's syndrome (trisomy 21) appears in pathology textbooks (Eggston & Wolff, 1947, Schuknecht, 1974), except for that in Friedmann's textbook (1974) Friedmann introduced Wright's study (1969) on the temporal bones in four cases of mongolism (trisomy 21), which study described inflammatory changes in the middle ear, though no cochlear abnormality was found

Johnsson (1971), using a surface-preparation technique on the temporal bone from a 4-month-old infant with Down's syndrome, reported that the lateral bony semicircular canal was malformed and shaped like a pouch, open to the vestibule The ampulla too was malformed and there was a stenosis of the membranous semicircular duct

In this report, we present the results, including dimensional measurements, of a comparative study on temporal bones from patients who had Down's dyndrome and from controls

This study was partly supported by NIH grant PO1-NS-10940

METHODS

The four pairs of horizontally sectioned temporal bones from patients with Down's syndrome were compared with 15 pairs of temporal bones from infants of similar age range (4 cases, 1 month of age, 4 cases, 2 months, 2 cases, 4 months, 1 case, 5 months, 1 case, 6 months, 2 cases, 7 months, and 1 case, 8 months) Most of the bones were from patients with infections, but none was involved with any developmental bony structure disease or anomaly

The lengths of the cochlear spirals were studied by the graphic reconstruction method (Guild, 1921, Schuknecht, 1953) Comparison of the vestibular apparatuses was done by dimensional measurement of the temporal bones in horizontal sections The measurements taken were the greatest diagonal distance between the medial bony wall of the vestibule and the most lateral bony wall of the lateral semicircular canal (measurement A), the diagonal width of the central bony trabecula enclosed in the lateral semicircular canal (measurement B), and the size of the bony semicircular canal at the most lateral part of the crus (measurement C) The comparative data analyses were done by t test (pooled variance method) (Winer, 1962)

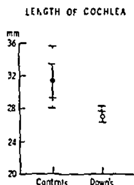


Fig 1 This figure shows the comparison of lengths of cochleas based upon graphic reconstruction between Down s syndrome and controls Solid lines indicate standard deviations and dashed lines indicate the ranges of sample distribution

CASES OF DOWN S SYNDROME

Case 1

A 2 month old infant clinically showed epicanthal folds, a simian line on the left hand, flat facies, abnormal spaces between the first and second toes of both feet, short palate, clinodactyly of the fifth digit of the hands, flattened occiput and bridge of nose, and low set pinnas Chromosomal analysis of the peripheral leukocytes confirmed the diagnosis of trisomy 21 The infant was observed to be responsive to stimulation *The infant's mother* was noted to be very deaf, clinically

Case 2

Simian lines on the hands, epicanthal folds, and flat facies characterized this 1½ month old infant Chromosomal analysis of the peripheral leukocytes confirmed the diagnosis of trisomy 21

Case 3

This 4-month old infant showed a mongoloid slant to the eyes prominent epicanthal folds, low set pinnas, simian lines on the hands, and clinodactyly of the fifth digits of the hands Chromosomal analysis was not diagnostic due to the viability of the cells in the specimen The infant was clinically active and alert *The mothers* pregnancy was complicated by influenza characterized by fever, chills, and aches in the first trimester

Case 4

At 3 months of age, this infant demonstrated clinically a lateral upward slant of the palpebral fissures, low set pinnas, and hypertelorism The infant was described clinically as atypical Down s syndrome, but no chromosomal analysis was done The Moro reflex was described as poor *The mother described an illness characterized* by headaches, dizziness, and emesis during the first trimester

No clinical abnormalities of the tympanic membranes were reported in any of the four cases All four cases were examined by autopsy

RESULTS AND DISCUSSION

Cochlea

By comparing the lengths of cochleas based on graphic reconstructions, it was obvious that the temporal bones from patients with Down s syndrome had shorter cochlear spirals than those of control ears (Fig 1) However, there was no profound difference in the width of the basal coils of the cochleas (in the mid modiolar sections) According to Sando et al (1975) the mean length of the cochlear spiral from 6 newborn infants was 31 73 mm In our control series of normally developed infant temporal bones, the spiral length was 31 4±2 1 mm whereas it was 27 5±0 9 mm in four pairs of temporal bones from infants with Down s syndrome When this set of data was analysed by a *t* test, the difference was found to be significant *($p<0$ 01, two-tailed)*

Other than the above described finding, the cochlear end organ and supporting structures were found to be properly developed and not degenerated (even though the existence of post mortem change affected the accuracy of the evaluation) No anomalies were found The slightly ectatic view of the scala media in the apical turn was seen in three ears and a slightly *hypogenic spiral ganglion* was noted in the



Fig 2 This photomicrograph exhibits a widened perilymphatic space of the utricular compartment and lateral sem circular canal in a temporal bone of a patient with Down s syndrome The posterior semicircular canal looks normal A central marrow space of the incus can be seen H & E staining ×9 5

basal turns of four ears however, these findings may not be true pathological findings No cystic stria vascularis was found A relatively wide opened cochlear aqueduct was seen in five ears in this present series and two of those ears each showed a large sized vena aqueductus cochlea enclosed in individual bony canals

In two ears a superiorly located inner orifice of the internal auditory canal and somewhat narrow, funnel shaped distal end were found Contrariwise the distal portion of the internal auditory canal was slightly distended in two other ears of this series compared with those of controls

Vestibular structures

A variety of anomalous configurations of the utricular space lateral semicircular canal, and associated structures together with end organ anomalies have been reported in the different forms of other trisomy cases Similarly, in this present series the majority of abnormalities were found in the vestibular apparatus

A widened utricular space and semicircular canal (and ampulla) were found in two pairs of temporal bones (Fig 2) whereas another two pairs in this series showed only slight widening The utriculo-endolymphatic valve was well developed in six ears whereas the other two ears showed unclear structural differentiation Most of the ears in this series had large endolymphatic sinuses The endolymphatic duct and sac were normally developed and clean but in three ears some precipitate was seen in the region

The abnormal configurations found in this series resembled in nature (but in different degrees) those previously reported in the temporal bones with other forms of trisomy sy



Fig 3 Photomicrograph showing an enlarged bony posterior ampulla that contains the membranous posterior semicircular duct The posterior semicircular duct lacked an independent bony semicircular canal The rugous portion of the endolymphatic sac contains precipitate including cellular components H & E staining, ×21

However, contrary to the findings in other forms of trisomy vestibular end organ anomalies (such as flattened crista or irregularly shaped macula) were not obvious in the present series of temporal bones with Down s syndrome

One significant finding in a pair of temporal bones in this series was a severe hypogenesis of the posterior semicircular canal In that case the posterior membranous semicircular duct was located within the enlarged bony posterior ampulla and no independent bony semicircular canal has developed (Fig 3) No abnormal nar

rowing of the membranous semicircular duct, as reported by Johnsson (1971), was seen in this series. Cystic configuration in the dark cell area was found in one pair of temporal bones.

In trisomy, the most frequently malformed structures are the lateral semicircular canals (Altmann, 1953). Sando et al. (1975) also reported that 10 out of 14 temporal bones with trisomy 13 showed some anomaly in the horizontal semicircular canal. In order to evaluate the structural framework development, we measured the greatest distance between the medial wall of the vestibule and the most lateral edge of the circle of the lateral semicircular canal (measurement A) in horizontal sections and compared those measurements between Down's syndrome cases and controls. This measurement A in Down's syndrome cases was found to be smaller than that in control infant temporal bones (Fig. 4). When a *t*-test was used for the analysis, the difference was significant ($p<0.01$, two-tailed).

In order to further reinforce this observation, measurement A in 70 adult temporal bones (random age, without any bone disease or structural anomaly) was applied to this comparison study in order to minimize variances caused by different cutting planes. These 70 adult temporal bones were selected on the basis of their being sectioned in a cutting plane similar to that in which the four pairs of temporal bones from infants with Down's syndrome were sectioned. Only one out of the 70 cases had 7.17 mm in measurement A, all others had more than 7.39 mm. On the other hand, in the temporal bones from patients with Down's syndrome, the largest value obtained in this measurement A was 7.03 mm.

Fig. 4 also displays the results of measurement B. A *t*-test showed that the difference between the two groups to be significant ($p<0.01$, two-tailed). The smaller values obtained in measurement A indicated the overall underdevelopment of the lateral semicircular canal framework, however, the concomitant existence of small values for me[illegible] that the vestibular pe

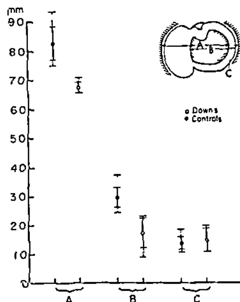


Fig. 4. This figure demonstrates comparisons of measurement A (the greatest distance between the medial wall of the vestibule and the most lateral edge of the lateral semicircular canal), measurement B (the width of central bony trabecula), and measurement C (the maximum width of the most lateral crus of lateral semicircular canal in horizontal section), between Down's syndrome cases and controls. Solid lines show ranges of standard deviations and dashed lines indicate the ranges of sample distribution. Note the clear differences in comparisons of measurements A and B.

lymphatic spaces were not necessarily narrow. Some of those vestibular areas were found to be wider than those in controls. However, no significant difference was found by a *t* test ($p>0.05$, two-tailed) between the two groups when the size of the most lateral crus of the bony canal (measurement C) was compared.

The measurement was not done on the vertical canals, however, the superior semicircular canal appeared the one least involved developmentally in this Down's syndrome case series.

No anomalous configuration was seen in the sacculus.

Middle and external ear

A varying amount of mesenchymal tissue was seen in all Down's syndrome ears. Three ears had infections, with thickened mucosa (inflam[illegible] infiltrated) and exudate. Also, a [illegible] of the central marrow s[illegible]



Fig. 5. This photomicrograph exhibits a slight deformity of stapes superstructure (distorted crura) found in a temporal bone from a patient with Down's syndrome. H & E staining, ×24

incus (and to some extent that of the malleus) was observed. A deformity of the stapes superstructure was found in one ear in which slightly distorted crura were found (Fig 5) Also, in that particular ear, a major portion of the stapedius muscle was exposed to the middle ear, and the pyramidal eminence was somewhat underdeveloped.

An obtuse angle of the facial genu was seen in one ear, and in another one we noted an exceptionally large vessel together with the facial nerve The facial nerve itself and the geniculate

ganglion were slightly hypoplastic in four ears, but otherwise not pathological

Tympanic membranes and external auditory canals looked intact

ACKNOWLEDGEMENTS

We would like to express our gratitude to the Dept of Pathology, Baylor College of Medicine, for their providing us with the temporal bone materials from Ben Taub General Hospital (Dr H -S Kim)

RESUMÉ

Une étude comparee a ete réalisee sur quatre paires d os temporaux de patients presentant le syndrome de Down (trisomie 21) et quinze paires d os temporaux d autres enfants du même age Les reconstructions hélicoidales ont montré que les longueurs des cochlees etaient légerement plus courtes dans les os temporaux avec le syndrome de Down que ceux des controles En se basant sur les mesures dimensionnelles, on a trouvé une anomalie du developpement vestibulaire

ZUSAMMENFASSUNG

Vier Schläfenbeinpaare von Patienten mit Down Syndrom (Trisomie 21) wurden mit 15 Paaren von Schlafenbeinen von anderen Säuglingen gleicher Altersgruppen verglichen Spirale Wiederherstellungen zeigten, daß die Länge der Cochlea etwas kurzer war in Schlafenbeinen mit Down Syndrom als die der Kontrollschlafenbeine Aufgrund dieser Ausmessungen wurde eine vestibulare Entwicklungsmißbildung gefunden

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DISCUSSION

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A METHOD FOR DETERMINING CORTICAL AUDITORY THRESHOLDS IN GUINEA PIGS

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Abstract A method for measuring evoked cortical potentials to acoustic stimuli through the intact dura mater of the guinea pig without computer averaging was studied After exposure of the dura overlying the auditory cortex on the left side thresholds to acoustic stimuli presented to the right ear were determined with a double tipped silver electrode by visual detection on an oscilloscope The thresholds obtained are compared with other methods of determining audibility levels in the guinea pig With relatively simple instrumentation this method provides rapid and accurate determination of the audibility curve

Many methods have been developed to measure the auditory thresholds in guinea pigs Monitoring of the Preyer reflex (Horton, 1933) and classical behavioral conditioning (Gerstner, 1942) have significant limitations Behavioral suppression techniques (Anderson & Wedenberg, 1965, Miller & Murray, 1966, Heffner et al, 1971) have proved to be reliable indices of auditory sensitivity although they require considerable time

Objective physiological methods to assess auditory function were first applied to the end organ and eighth nerve by measuring the a c cochlear potential and the compound action potentials The cochlear potential was investigated with electrodes on the round window and subsequently with differential intracochlear electrode techniques Recently, the development of new techniques has resulted in a shift of interest to the more central parts of the auditory system Audibility curves based on evoked responses from the inferior colliculus compare very favorably with those from behavioral suppression techniques (Makishima et al, 1975) Additional information has been obtained by monitoring responses from the auditory cortex Kern et al (1969) Hattori & Shoyama (1970) and Djalilian & Cody (1973) have shown the usefulness of monitoring evoked responses from the temporal cortex Their method involves placing electrodes directly on the dura mater with response detection by computer averaging The need for computer averaging for determining threshold was obviated by Walloch (1975) who removed the dura mater and placed the electrode directly on the pia mater of the guinea pig

A study of a method for measuring evoked cortical potentials through the intact dura mater of the guinea pig without computer averaging is presented The surgical technique is simple and direct The procedure provides a quick and accurate method of obtaining auditory threshold sensitivity

SUBJECTS AND PREPARATION

Adult colored guinea pigs, weighing between 200–250 g, were used in this study Each animal was anesthetized with sodium pentobarbital 40 mg/kg of body weight administered intramuscularly *A tracheal cannula was inserted*, and the animal was mechanically ventilated After being

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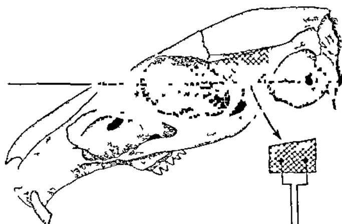


Fig 1 The skull of a guinea pig is illustrated The shaded area represents the dura which is exposed that overlies the auditory cortex A bipolar electrode with an intertip distance of 5 mm is gently touched to the dura

secured in a head holder, the skull was positioned so that the foramen ethmoidale and the center of the external auditory canal were in the horizontal plane The soft tissue was elevated from the skull to expose the greater part of the calvarium The left squamous bone, which overlies the auditory cortex, was removed with motor-driven burr and curette The boundaries of the squamous bone are the parietal squamosal suture line superiorly, the horizontal-0 line inferiorly, the frontal 0-line anteriorly, and the junction of the squamous bone and tympanic bulla posteriorly (Fig 1) (Rossner, 1965) The dura was exposed with care to avoid abrading or penetrating it The superior part of the right external auditory canal was incised about 3 mm lateral to the tympanic ring for insertion of a sound tube

APPARATUS AND PROCEDURE

The sine wave output from an oscillator was formed into tone bursts with 5 msec rise–decay time and 50 msec duration by passing it through an electronic switch Tone bursts were amplified and transmitted to a Teledynamics TDH-49 earphone Stimulus frequency was monitored with a frequency counter, and intensity was controlled with a decade attenuator Click stimuli consisting of 0 1 msec square wave pulses were occasionally used

A closed sound system was formed The ear phone was sealed to the sound tube A probe tube of the microphone (Bruel & Kjaer condenser microphone model 4134), which had been calibrated prior to experimental use with another condenser microphone (Bruel & Kjaer, model 4138), was passed through the sound tube The microphone was sealed to the sound tube The sound probe tube was placed as close as possible to the tympanic membrane, and the acoustic stimuli at the probe microphone were converted to SPL at the tympanic membrane and expressed as dB re 20 μN/m^2

A bipolar silver electrode with tip diameters of 1 mm and an intertip distance of 5 mm was gently touched to the dura A grounding cable was attached to the neck muscles The preparation was allowed 10 min to stabilize before data were collected The signals detected from the auditory cortex were amplified 1 000 times (band pass 1 0 Hz to 1 0 kHz), and the response was displayed on an oscilloscope the sweep of which was synchronized with the tone bursts A procedure similar to the method of limits was used to determine a visual detection level (VDL) threshold for the evoked responses from the auditory cortex (Kayser & Legouix, 1963, Zeigler, 1964) Thresholds were obtained at 17 frequencies in successive 1/3 octave steps between 0 5 and 20 0 kHz

All testing was done in a sound attenuated booth A 60 W light bulb was placed above the animal, and the ambient air temperature was maintained at approximately 37°C A YSI model 73A telethermometer was coupled with a heating pad, and the rectal temperature was maintained at 37±1°C

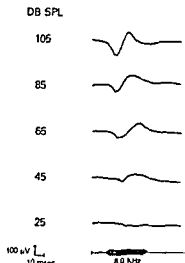


Fig 2 Evoked reponses from the auditory cortex for 8 kHz tone bursts. The evoked response amplitude diminishes as the SPL is attenuated The VDL threshold of this example is 25 dB SPL.

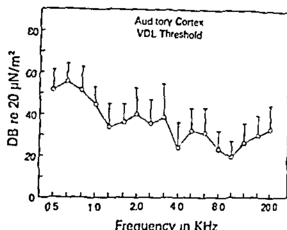


Fig 3 The average VDL thresholds at 17 test frequencies are illustrated ($n=10$) Vertical range bars indicate 1 S D above the mean The most sensitive frequency tested is 10 kHz (19 5±7 4 dB SPL)

RESULTS

With this technique, acoustically evoked responses were consistently recorded from the auditory cortex A typical VDL threshold sample for 8 0 kHz tone bursts is shown in Fig 2 The evoked response amplitude decreases as the SPL is attenuated In this example, the threshold is approximately 25 dB SPL There is close correlation between stimulus intensity and onset and peak latency With high intensity levels of 125 dB SPL at 8 0 kHz, the onset latency ranges from 12–18 msec (13 7±2 3), and the peak latency ranges from 18–30 msec 25 0±4 0) As the intensity is attenuated to threshold levels (25 dB SPL), the onset latency ranges from 25–30 msec (28 0±2 4), and the peak latency ranges from 40–48 msec (43 5±4 1)

The average VDL thresholds at each test frequency from 10 animals are presented in Fig 3 The vertical range bars represent one standard deviation above the mean The most sensitive frequency tested is 10 0 kHz (19 5±7 4 dB SPL) The intensity function of the response was also determined and at 125 dB SPL had a maximal amplitude of 152±29 4 µV for 2 0 kHz and 140±12 2 µV for 8 0 kHz and 110±56 5 µV for 16 0 kHz The amplitude decreases as the stimulus intensity is attenuated

DISCUSSION

The importance of the guinea pig as an experimental subject for auditory research is well documented, and much is known about the physiological and morphological aspects of its auditory system Reliable methods of measuring threshold and suprathreshold auditory re sponses under a variety of experimental circumstances are of considerable practical importance

This method utilizes a relatively simple surgical procedure It depends on visual detection of the response rather than computer averaging By maintaining the integrity of the dura, the method is applicable to prolonged and repeated measurements as well as acute experimental conditions The use of the bipolar electrodes allows stable data collection from multiple sites on the dura overlying the auditory cortex within the described surgical landmarks Bipolar electrodes with a 5 mm intertip distance have the ability to sample large areas of the auditory cortex (Kayser & Legouix, 1963, Zeigler, 1964) Indeed, the intertip distance of these electrodes approximates the diameter of the auditory cortex in the guinea pig, and this method obviates the sampling of responses from various positions on the cortex as described by others (Hattori & Shoyama, 1973, Walloch, 1975) The relative size of the intertip distance to the size of the auditory cortex in the guinea

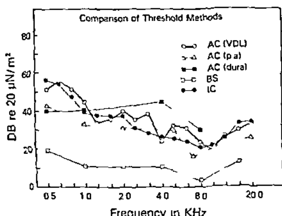


Fig 4 Comparison of threshold methods in the guinea pig AC (VDL) Cortical response by the present method, AC (pia) Cortical response on pia mater without computer averaging by Walloch (1975), AC (dura) Cortical response on dura mater with computer averaging by Djalilian & Cody (1973), BS Average of three behavioral curves by Anderson & Wedenberg (1965) Miller & Murray (1966) and Heffner et al (1971), IC Responses from inferior colliculus by Makishima et al (1975)

pig precludes the use of this method in tonotopic analysis of the auditory cortex

The thresholds obtained with other methods of determining audibility levels in the guinea pig are compared in Fig 4 The three behavioral suppression procedures have been averaged and presented as a single behavioral curve (Anderson & Wedenberg, 1965, Miller & Murray, 1966, Heffner et al, 1971) The cortical evoked response curves obtained with computer averaging (Djalilian & Cody, 1973) and without computer averaging (Walloch, 1975) and the evoked response curve from the inferior colliculus (Makishima et al, 1975) are also presented The similarity of the cortical evoked threshold curves obtained with methods described by others (Djalilian & Cody, 1973, Walloch, 1975) and this method is apparent in Fig 4 The correlation of the evoked response curve from the cortex with the evoked response curve from the inferior colliculus (Makishima et al, 1975) and with the behavioral audibility curve is of great interest Thresholds at the auditory cortex show spectral sensitivities which parallel the behavioral curve The strong correlation between these electrophysiological thresholds and the behavioral thresholds make this evoked response method particularly useful

The use of a general anaesthetic does have an effect on evoked responses from the brain Most general anaesthetics cause a slight loss in the amplitude of evoked responses, but usually the latency and duration of the response does not differ from the unanaesthetized state (Celesia & Puletti, 1971; Nakai et al, 1965) In our study of this method, 40 mg/kg of body weight of sodium pentobarbital was used for general anaesthesia Since the animal is immobilized and the middle ear muscles are inactive, the stimulus can be specified with precision The level of central nervous system depression can be maintained fairly constant General anaesthesia decreases neural noise and increases the signal to noise ratio so that the response can be detected visually at sound pressure levels that approximate threshold determinations

Measurement of evoked responses from the inferior colliculus may be made in the same animal preparation with this cortical technique By applying this method along with methods for measuring responses at the inferior colliculus and methods of measuring cochlear potentials, the investigator is able to sample different levels of the auditory system in the same animal preparation

The ease of the surgical technique, the use of relatively simple instrumentation and the rapid and accurate determination of the audibility curve suggest this method as an alternative to more complex methods of determining auditory sensitivity at the cortex Its application in the study of various problems in the auditory system is recommended

RÉSUMÉ

Nous avons étudié une méthode qui permet de mesurer des potentiels corticaux provoqués par des stimulations acoustiques au travers la dure mère intacte d un cobaye sans « computer averaging » Apres l'exposition de la dure mere sur le cote gauche, nous avons determiné avec une électrode d argent à doubles pointes, le seuil à des stimulations acoustiques presentées à l oreille droite par détection habituelle sur un oscilloscope Les seuils obtenus sont comparés à ceux trouvés par d autres

méthodes, qui déterminent les niveaux d audibilité chez le cobaye avec une instrumentation relativement simple Cette méthode permet une détermination rapide et précise des courbes d audibilité

ZUSAMMENFASSUNG

Es wurde eine Methode erforscht, die ohne Durchschnittsberechnung durch Komputer, die evozierten Rinden spannungen auf akustischen Reizmitteln durch die un verletzte Dura mater des Meerschweinchens mißt Nach der Bloßstellung der Dura, die über der Auditivrinde auf der linken Seite liegt wurden Schwellenwerte fur die akustischen Reize auf das rechte Ohr mit einer doppel spitzigen Silberelektrode durch visuelle Ermittlung auf einem Oszilloskop festgestellt Die erzielten Schwellenwerte werden mit anderen Methoden der Feststellung der auditiven Stufen im Meerschweinchen verglichen. Mit verhältnismäßig einfacher Gerätschaft ergibt diese Methode eine schnelle und genaue Feststellung der Audibilitätskurve

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DISCUSSION

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VESTIBULAR SYNDROME AND VASCULAR ANOMALY IN THE CEREBELLO-PONTINE ANGLE

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Abstract During the investigation of patients presenting symptoms related to the Vth and VIIth nerves, frequently associated symptoms relevant to the VIIIth nerve were found, such as tinnitus, disequilibrium or vertigo On the other hand, there also came to our attention patients whose major complaints were of VIIIth origin especially of the vestibular component, and who occasionally presented associated symptoms of Vth or VIIth nerve involvement Following the successful treatment of trigeminal neuralgia and hemi facial spasm by liberation of the Vth and the VIIth nerve from the mechanical irritative lesion frequently a vascular loop anomaly, it was postulated that the same cause could be responsible of tinnitus and vertigo, secondary to irritation of the VIIIth nerve, in some cases The clinical picture of VIIIth nerve involvement in the posterior fossa either isolated or associated with facial pain or hemi facial spasm, is presented together with the results of surgical treatment *in 5 cases*

Mechanical irritation of the Vth and VIIth nerves by a malformation or anomaly of osseous, vascular, arachnoidal adhesions or a tumor can induce symptoms of an irritative nature, such as facial neuralgia or hemi-facial spasm Dandy (1934) was the first to describe a compression of the Vth sensory root with the posterior fossa as the cause of trigeminal neuralgia In a subsequent review of Dandy's series of 160 acoustic neurinomas, Revilla (1948) found 16 patients who had typical "tic douloureux" and hemi-facial spasm Gardner & Miklos (1959) showed that the paroxysm of the hemi facial spasm, like trigeminal neuralgia, may be stopped immediately by non traumatic manipulation of the nerve root without impairment of function

In hemi facial spasm, Ehni & Woltman (1945) stated that gross lesion of the facial nerve was causing twitching of the face, almost or actually indistinguishable from the twitching of the so called "cryptogenic" hemi-facial spasm It is interesting to note that in their series of 106 cases, 15 patients also presented deafness on the spasm side

Other authors, such as Olivecrona (1949), Olive & Svien (1957), Gardner (1962), Revilla (1948), Taarnhoj (1952), Bjerrum & Thornval (1959), Gardner & Sava (1962), presented further evidence concerning the cause of facial hemispasm by tumor or vascular anomalies in the posterior fossa Janetta (1975), has reported his experience concerning the treatment of trigeminal neuralgia and hemi facial spasm by decompression of the Vth and VIIth nerves of anomalous arteries, adhesions or tumors One of the authors (Provost & Hardy, 1970) has already reported the results of treatment of trigeminal neuralgia Results in patients investigated and operated for hemi facial spasm have also been presented by the authors (Bertrand et al, 1976)

During the investigation of patients with symptoms pertaining to the Vth and VIIth nerves, there were frequently associated symptoms relevant to the VIIIth nerve, in particular, deafness and tinnitus for the cochlear portion and disequilibrium or vertigo in relation to the vestibular portion Anomalies of VIIIth nerve function were occasionally found On the other hand, patients investigated for symptoms whose major complaints were of VIIIth nerve origin frequently had symptoms of Vth or VIIth nerves The investigation usually demonstrated

electrophysiological abnormalities We then postulated that the same causes may be responsible for cochlear or vestibular nerve symptoms

It is our impression that abnormal stimulations of the cochlear or vestibular nerves, whether by a tumor, abnormal vessels, or arachnoidal adhesions, can induce symptoms relevant to these nerves and that removal of the abnormality could result in the disappearance of the symptoms Janetta (1975) has reported 8 cases of hyperactive dysfunction of the VIIIth cranial nerve treated by neuro-vascular surgical decompression

A clinical picture investigation and surgical treatment of 5 patients are now presented (Table I)

METHOD OF INVESTIGATION

Audiometric evaluation

The audiometric assessment included tonal audiometry for the frequencies of 125 to 8 000 of the SRT, of vocal audiometry of SISI, ABLB, Bekesy, tone decay and impedance audiometry studies This complete battery of s was not carried out on all of the patients

Vestibular investigation

The function of the vestibular apparatus was determined by recording ocular movements by ENG We have already reported our technique in detail (Bertrand, 1970) We routinely studied ocular motricity, spontaneous nystagmus with the eyes opened, eyes closed and lateral gaze, positional stimulation in 7 different positions, caloric stimulation at 30° and 44° and, when necessary, at 0°C When irrigation was contraindicated, the caloric stimulation was done according to the technique of Dundas Grant Rotatory stimulation using pendular and angular acceleration was done as an additional test in certain cases

Study of the Vth and VIIth nerve reflexes

The study of the trigeminal and facial reflexes was done by mechanical and electrical stimulations of both nerves according to the technique already described by Molina et al (1976) The peripheral conduction of the facial nerves was also evaluated

Radiological evaluation

In cases presenting a possibility of pathology of irritative nature at the cerebello pontine angle, an angiographic study was made with an incidence allowing us to demonstrate adequately the vascularization at the level of the internal auditory meatus and to detect the possibility of a vascular anomaly In certain cases, PEG was used when we believed that there was an obstructive or tumoral lesion in the internal auditory meatus

SURGICAL INDICATIONS

The main indication for surgery was based on the intensity of the symptoms in regard to the Vth, VIIth or VIIIth nerves All 5 patients presented symptoms which were of such intensity that they could not carry out regular activities or were incapable of working The results of the investigation carried out were positive in confirming an organic lesion of the nerve and the lesion side

CASE HISTORY

Case No 1

C B This 45 year-old woman had two vertiginous spells in 1959, without any associated cochlear symptoms In 1971, she again had a severe vertiginous crisis which gradually increased in intensity and frequency to 5 to 6 times a month This was her major complaint She also presented a disequilibrium between the dizzy spells which was persistent She had no loss of hearing Moreover, in the last 6 months she noticed numbness of the left lower lip, suggesting Vth nerve involvement The pre and postoperative trigemino facial reflexes are demonstrated in Fig 1*a*, *b*

On September 12th, 1974, a left posterior fossa approach was undertaken Arachnoid

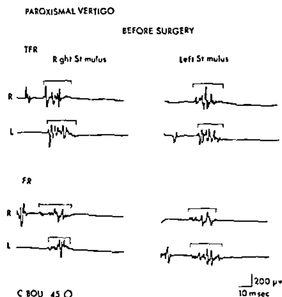


Fig 1a Case No 1 Trigeminal facial reflexes (TFR) demonstrated pre operatively increased latency and diminished amplitude of the left R1 response Increased latencies of both R2 were obtained after left trigeminal and facial nerves stimulations

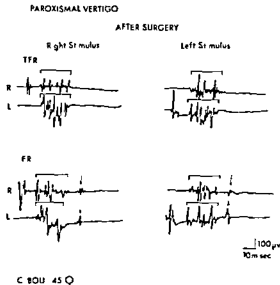


Fig 1b Post-operatively there are bidded simetrical latencies and amplitude of R1 and R2 following trigeminal and facial stimulations

adhesions were found on the acoustico-facial complex After dissection of these arachnoid adhesions, a large arterial loop adherent to the facial nerve and the cochleo vestibular nerve was seen Dissection under microscope allowed us to observe this arterial loop passing between the VIIth and VIIIth nerves and adhering to the inferior portion of the vestibular nerve This arterial loop was mobilized away from the nerve There was no surgical complication The patient was observed at regular intervals and after 23 months, she had no recurrence of her symptoms

Case No 2

O S This 48-year-old woman had complained for the last 6 months of an anti-clockwise rotatory vertiginous crisis lasting up to 3 hours, accompanied by a vague sensation of pain on the right side of the head She also noticed twitching of the peri buccal muscles on the right side compatible with a localized hemi facial spasm This was associated with a non-rotatory disequilibrium On examination, spontaneous nystagmus towards the right side was present Reduced sensation on the right side of the face and of the right external auditory meatus were found Thermic sensation was also diminished in the right ophthalmic division Twitching of the right side peri buccal muscles could be easily observed, being compatible with a localized hemi-facial spasm

On September 26th, 1974, a sub-occipital retro-mastoid approach was undertaken The exploration revealed an arterial loop tightly adherent to the inferior part of the VIIIth nerve corresponding to the inferior vestibular fibres and the VIIth nerve anteriorly The arterial loop was visualized encased between these two nerves It was gently separated and moved away from the nerves after opening the arachnoidal membrane

There was no complication in the immediate postoperative period 22 months after the surgery, this patient is free of the vertiginous



Fig 2 Case No 3 The VIIth and VIIIth nerves are separated by a loop of the internal auditory artery Mobilizat on of this artery from this abnormal localizat on was achieved by interposing gelfoam be tween the two nerves and the arterial loop

toms and the twitching of the peri buccal muscles has disappeared

ase No 3

A This 53 year-old man had a vertiginous rotatory crisis with nausea and vomiting associated for the last 10 years with bilateral tinnitus and bilateral hypoacusis One year ago, he began to suffer from pain in the right cheek, compatible with a neuralgia of the second trigeminal branch On neurological examination hypoalgesia of the ophthalmic and maxillary branches of the right trigeminal nerve were found

A left sub occipital exploration was performed on October 22nd, 1974 Through a thin trans parent arachnoidal membrane several arterial loops could be visualized After opening the arachnoidal membrane, an arterial loop was visualized passing between the VIIth and VIIIth nerves (Fig 2), making several turns around these nerves An artery, corresponding to the internal auditory artery could be seen originating from one of the loops After dissection of the arachnoidal membrane, the arterial loop was mobilized away from its position between the VIIth and VIIIth nerves The immediate post operative period was uneventful and 21 months following surgery, the patient is symptom free

Case No 4

R W This 28 year old male patient had several minor cranial traumata, although never accom panied by loss of consciousness but only slight obnubilation for a few seconds For four years, he had complained of vertigo which he described as an intense disequilibrium with a tendency to fall This was associated with headaches and pain in the left ear canal He also had occasional ly some tinnitus of a pulsating nature associated with a fluctuating hearing loss on the left side, and in addition, a sensation of numbness in his face Because of the persistence and intensity of the symptoms, a surgical exploration was decided on

Via a sub occipital appraoch, a vascular loop anomaly was found encroaching upon the ves

tibular and cochlear nerves The vessel was mobilized away from the VIIth and VIIIth nerves The postoperative period was uneventful and 18 months later, he had no recurrence of symptoms

Case No 5

B L This 48 year-old man experienced a sudden noise in his right ear 2 years before consultation This was followed by deafness, nausea and disequilibrium The sensation of deafness disappeared after a few weeks but the tinnitus and disequilibrium persisted and were intensified by changing position A few months later, the disequilibrium had so increased that he was obliged to stop work He then started to have pain in the right ear and a sensation of fullness or pressure on the right side of the head

Clinical examination demonstrated hypoesthesia and hypoalgesia of the entire right side of the face and in the right auditory meatus Cysternography was negative Nevertheless, because of the persistence of the syndrome and due to the fact that this patient could not work for the last 2 years, we proceeded to a surgical exploration

Via sub-occipital exposure of the cerebellopontine angle, an abnormal arterial loop was found in contact with the Vth and VIIth nerves and the vestibular and cochlear portion of the VIIIth nerve The arterial loop was mobilized away from the nerve by interposing a piece of gelfoam

The symptoms gradually disappeared during the months following surgery and the patient has resumed his work There were no symptoms at the last control, 19 months after surgery

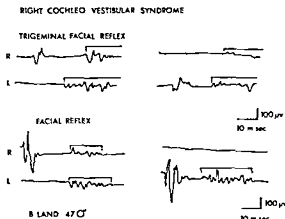


Fig 3 Case No 5 Preoperative trigemino-facial reflexes demonstrate an increased latency and diminished intensity of both the mono and polysynaptic responses on the right side The facial reflexes also present increased latency responses diminished amplitude on the right side after right stimulation and absent response on the right following left stimulation

DISCUSSION

In the presence of vestibular or cochlear symptoms, one must consider the possibility of an irritative or compressive mechanism on the vestibular nerve in its trajectory in the posterior cranial fossa between the internal auditory meatus and the brain stem This possibility is frequently neglected in favour of a peripheral origin at the level of the labyrinth or a central origin at the level of the brain stem From the clinical point of view, one must suspect a lesion of the cerebello-pontine angle when a vertiginous syndrome is accompanied by signs involving other cranial nerves that of the facial nerve manifested by hemi facial spasm or otalgia, that of a trigeminal nerve by a facial neuralgia There exist particularities of the audiometric and vestibular tests which can orientate the lesion at the level of the posterior fossa

Special audiometric test findings will tend towards a retro-cochlear rather than a cochlear pathology, even though in many patients these tests prove normal Vestibular tests, on the other hand, frequently demonstrate an ipsilateral hypoexcitability Abnormal spontaneous or positional nystagmus rarely occurs Finally, the trigemino-facial reflexes study demonstrates an alteration of the latencies secondary to irritation and compression of the trigeminal and facial nerves with abnormal conduction responses in the trajectory between the internal auditory meatus and the brain stem

These studies are complemented by an angiography and air study in order to determine the

Table I Summary of symptoms, investigations and surgical findings

Case No	Sex	Age	Symptoms	Preoperative investigation: Hearing	Vestibular	Facial Reflexes	X rays
1 C B	♀	45	VIII Vertigo V Numbness of face	Normal	Spontaneous and hyporeflective	Abnormal latency on left side	Angiography normal PEG abnormal
2 O S	♀	48	VIII Vertigo VII Facial spasms V Numbness of face	Normal	Spontaneous and hyporeflective	Decreased ipsilateral response	PEG abnormal
3 R A	♂	53	VIII Vertigo Hypoacusis V Facial neuralgia	Normal	Spontaneous and hyporeflective	Decreased ipsilateral response	Not done
4 R W	♂	28	VIII Disequilibrium Tinnitus VII Otalgia	Normal	Hyporeflective	Increased latency and decreased intensity	Angiography abnormal arterial loops
5 B L	♂	48	VIII Vertigo Tinnitus VII Otalgia V Hypo-esthesia	Neuro sensorinal hearing loss with recruitment	Hyporeflective	Increased latency	Cysternography normal

...ssibility of a vascular anomaly or of an obstructive lesion in the cerebello pontine angle

It is not at all easy at the present time to establish a precise relation with the radiological study In fact, it is frequently 'a posteriori' that we can define in a precise manner the relation between the anatomical and radio logical findings Once the lesion of the posterior fossa is diagnosed and providing the symptom atology warrants it, surgical exploration of the cerebello pontine angle is indicated This ex ploration, done under the microscope, presents minimal risks in the hands of an experienced surgeon

In all of the 5 cases which we report the surgi cal exploration of the ponto cerebellar angle with liberation of the cranial nerve led to improvement which was sustained for periods varying from 19 to 23 months postoperatively

We are limiting our presentation to these 5 cases, not because of the excellent results obtained, but more to draw attention to the fact that it is possible to find anomalies of either vascular or arachnoidal origin capable of producing symptoms relevant to several cranial nerves in the cerebello pontine angle of the posterior fossa

From the results of these 5 patients operated to date, it appears that the cause of the vestibular or cochlear symptoms was the irritation of these cranial nerves by a vascular loop intimately attached to these nerves by arachnoidal ad hesions

In the case of trigeminal neuralgia as well as in the hemi facial spasm, anatomical study by means of electronmicroscopy demonstrated demyelinising lesions of the peri neurium which might cause short-circuiting of the electrical current, thus possibly causing abnormal nerve irritation and also slowing of the conduction of the facial reflexes This process may also be involved in the origin of vertiginous syndromes It is commonly known that tumors compressing the cranial nerves usually produce deficiency

toperative investigation			
aring	Vestibular	Facial reflexes	Surgical findings
rmal	No spontaneous normal calorics	Symmetrical and normal	Arachnoidal adhesions Abnormal vessel between VII and VIII
rmal	No spontaneous hyporeflective response	Not modified	Arachnoidal adhesions Abnormal vessel
rmal	No spontaneous hyporeflective response	Improved	Abnormal vessel
rmal	Not modified	Not modified	Abnormal vessel
t odified	Not modified	Not modified	Abnormal vessel

symptoms It is exceptional to note a vertiginous syndrome or hemi-facial spasm as a clinical manifestation of a tumor of the ponto-cerebellar angle In the investigation of these patients, the facial and trigeminal reflexes are important in determining the site of the lesion on the nerve Our experience of 5 patients with a surgically confirmed neuro-vascular anomaly and a follow-up of over 18 months of remission would seem to further confirm the possible neuro-vascular complex anomaly in the cerebello-pontine angle

RESUMÉ

Parmi les patients porteurs d une pathologie cochléo-vestibulaire, l investigation audiometrique et électro-nystagmographique chez 5 patients nous a orienté vers la possibilite d une pathologie rétrocochleaire Une étude angiographique de la fosse postérieure a mis en évidence une anomalie vasculaire chez les 3 patients Devant ces résultats, nous avons procedé à l'exploration chirurgicale de la fosse postérieure ce qui a permis de confirmer l existence d une artère anormale en contact immédiat avec le paquet acousticofacial La libération du nerf par eloignement de l'artère à son contact a resulté, dans tous les cas, à la disparition de la symptomatologie

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ATP ANWENDUNG BEI INNENOHRERKRANKUNGEN IN DER KLINIK UND IM EXPERIMENT

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Abstrakt I a ATP infusionen mit Glukose und Hyaluronidase haben bei 267 Pat mit verschiedenen Innenohrerkrankungen — zumeist Horsturzen — auch noch bei Spatfallen gute klinische Ergebnisse gebracht Zu letzt wurde in resistenten Fallen zusätzlich hyperbarer O_2 erfolgreich eingesetzt Der Versuch horsturzahnliche Bedingungen am Meerschweinchen als Modell zu schaffen mißlang, weil ein Horsturz beim Menschen nicht dem Hypoxiezustand beim Tier entspricht Solange der Nachweis einer ATP vermehrung in den Zellen der Cochlea nach i a ATP infusion nicht gelingt muß fur die unzweifelhaften klinischen Erfolge ein unspezifischer Mechanismus angenommen werden

Die Zahl der Patienten mit Horsturz nimmt dauernd zu Auch wir benutzten zur Behandlung die verschiedensten konservativen und chirurgischen Methoden, teils mit teils ohne Erfolg Da die Ätiologie des Horsturzes immer noch unklar ist, wird meist eine polypragmatische Behandlung angesetzt Unsere Erfahrungen an Patienten wiesen vor allem darauf hin, daß der Horsturz eine funktionelle vasculäre Innenohrstorung ist, wie es Calearo & Giordano (1959) Pfaltz (1960), Van Dishoeck (1966), Neveling (1967) Plester (1971) u a schon annahmen Die Durchblutungsstorung der Cochleagefäße ist sicherlich das Hauptmoment des Hörsturzes, obgleich wir wissen daß auch eine multiple Genese vorliegen kann oder sogar andere als primare Durchblutungsstörungen im Vordergrund stehen konnen Deshalb fanden vor allem Vasodilatantien Anticoagulantien und Gaben von energiereichen Phosphatgemischen in der Therapie Anwendung

Aus der angiologischen Forschung ist seit 1947 durch De Bakey et al bekannt, daß in ischaemischen Gebieten Vasodilatantien per os, i m oder i v einen unerwunschten Blutentzug, das „borrowing lending" genannte Phänomen erzeugen Um dies zu vermeiden, empfiehlt man wiederholte intraarterielle Infusionen Bei peripheren und zentralen Durchblutungsstorungen hatten sich i a ATP infusionen als erfolgreich erwiesen Unter Berucksichtigung der Faktoren

1 der Horsturz ist eine Durchblutungsstörung des Innenohres, und
2 die Art auditiva interna ist keine Endarterie (Hansen 1969) sondern zeigt Verbindungen zu Mittelohrgefaßen Demnach ist sie fur das Carotisblut erreichbar,

haben Morl et al aus unserer Klinik folgendes ATP Gemisch zur Hörsturztherapie eingeführt

80 mg Adenosintriphosphat
150 IE Hyaluronidase
8 g Glucose zu 50 ml physiologischer Kochsalzlosung

aufgefullt Mit Hilfe eines Dauerinfusionsgerates wird in die Art carotis in 30 min infundiert, und zwar jeweils auf der erkrankten Seite Der Einstich erfolgt nach Tranquilizer-Praemedikation und in geringer Lokalanaesthesie Täglich wird 1 × behandelt, in der Regel 5–10 Tage lang, auch Wiederholungen der Infusionsserie können erfolgen Morl et al haben während der Infusionen die zentrale Durchblutungssteigerung mittels dreier Verfahren gemessen, und zwar durch die Schadelrheographie, die Ophthalmodynamographie und die Pulsschreibung an

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DISCUSSION

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ATP - Infusionen – 267 Pat

	Rest od integr	Höranstieg gut	Höranstieg mäßig	Höranstieg ohne	gesamt
1	29	30	19	11	89
2	5	15	33	23	77
3	1	3	9	19	32
4	6	5	7	8	26
5	–	3	8	21	32
6	–	1	5	5	11

1 Hörsturz 3 Wo , 2 n 3 Wo. 3 n 1 Jahr 4 Menière
5 Progr Hypacusis int , 6 Toxische Innenohrschäden (Operationslabyrinthosen Gruppe)

Abb 2 Hörergebnisse unserer ATP Infusionen bei Hörstürzen und einigen anderen Innenohrerkrankungen

hirns funktionell nicht wirksam wird Diese Tatsache ist leicht an dem Verbleib von Kontrastmitteln, die zur angiographischen Darstellung der Hirngefäße verwendet werden, zu überprüfen So fand Hübner bei der Auswertung von 200 Carotisangiogrammen lediglich in 4 Fällen eine Darstellung der A basilaris über den Ramus communicans posterior

Umstritten bleibt weiterhin, ob das energiereiche ATP oder dessen Spaltprodukte ADP und AMP eine Stoffwechselverbesserung im ischaemischen Innenohr bringen können, oder ob es sich um einen unspezifischen Effekt auf das Gefäßsystem handelt, der mit einer durch Schädelrheographie, Ophthalmodynamographie und Arteriatemporalis-Pulsschreibung klinisch nachgewiesenen Blutflußerhöhung verbunden ist und somit eine Stoffwechselsituation im Innenohr schafft, welche die klinisch objektivierbaren Hörverbesserungen erklären kann Nach Vosteen ist das ATP nicht in der Lage, in eine Zelle infolge schlechter Membranpermeabilität einzudringen Es erfolgt ferner ein zu schneller Abbau des ATP durch Einstellung der örtlichen Gleichgewichtsverhältnisse

Um diese Frage möglichst zu klären, ahmten wir die klinische Situation im Tierexperiment an 58 Meerschweinchen nach

METHODIK

Die Meerschweinchen wurden in tiefer Urethannarkose nach Muskelrelaxation tracheotomiert und mittels einer Kleintierrespirationspumpe mit Luft künstlich beatmet Nach Freilegung der Bulla wurden die Mikrophonpotentiale von der Basalwindung der Cochlea nach Anbohren der Scala tympani und Einkleben einer Silberdrahtelektrode mittels Histoakryl abgeleitet und nach einer entsprechenden Verstärkung mit einem Differentialverstärker über einen Pegelschreiber auf Wachspapier übertragen Die für diese Versuche erforderliche indifferente Gegenelektrode befand sich in der Nackenmuskulatur Sichtkontrollen erfolgten an einem parallel geschalteten Oszilloskop Beschallt wurden die Tiere im freien Schallfeld mit automatisch unterbrochenen gefilterten Tonimpulsen von 1 800 Hz und einer Intensität von 80 dB Nach einer Vorregistrierung über einen Zeitraum von 15 min — gleiche Potentialhöhe vorausgesetzt — wurde das Verhalten der MP unter einer Hypoxämie von 3 min Dauer und die Wiederholung nach Weiterbestimmung registriert

Die Ausgangspotentialhöhe wurde auch bei mehrfacher Versuchswiederholung in Vorversuchen erreicht Im Hauptversuch wurde die apnoische Phase von 3 min wiederholt und es wurde einer Gruppe von 15 Tieren eine ATP-Infusion in die A carotis communis der operierten Seite unmittelbar vor der apnoischen Pause verabreicht Dazu wurden 5 ml der in der Klinik verwendeten und schon beschriebenen ATP-Stammlösung mittels einer Mikropipette mit einem Spitzendurchmesser von 20–30 μm injiziert Die Mikropipette wurde deshalb so klein gehalten, damit das Lumen der Arterie nicht ausgefüllt und der Blutstrom nicht blockiert wird

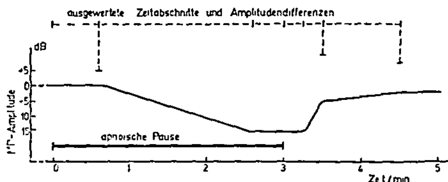


Abb 3 Amplituden Zeitdiagramm der MP-ableitung beim Meerschweinchen vor, während und nach einer Apnoe

Untersuchungsgruppen

1. 15 Tieren wurden 5 ml der klinisch verwendeten ATP-Lösung vor und während einer Apnoe verabreicht
2. 10 Tieren wurden 5 ml der klinisch verwendeten ATP-Lösung vor, während und nach einer Apnoe verabreicht
3. 9 Tieren wurden 5 ml einer ADP-Lösung vor, während und nach einer Apnoe verabreicht
4. 11 Tieren wurden 5 ml einer 40%igen Glukose-Lösung vor, während und nach einer Apnoe verabreicht

ERGEBNISSE

Die erhaltenen Werte, die mit der MP-Amplituden-Zeitverläufen der Vorversuche verglichen werden, zeigten keine statistisch zu sichernden Verbesserungen der Innenohrleistung während der Hypoxie und somit stehen unsere experimentellen Ergebnisse im Gegensatz zu denen von Faltynek & Vesely (1966), die durch intravenöse Verabreichung von ATP und AMP einen günstigen Effekt bei experimentellem Sauerstoffmangel oder nach einer 10-minutigen Lärmbelastung am Meerschweinchen beobachteten Zur statistischen Absicherung verwendeten wir den *F*- und den *t*-Test

Auf Grund dieser tierexperimentellen Ergebnisse möchten wir uns der Meinung von Vosteen (1961, 1973), Danilidis (1972), u a anschließen und glauben, daß es sich auch bei einer intraarteriellen Verabreichung von ATP um einen auf das Gefäßsystem unspezifisch wirkenden Effekt handelt, der klinisch genutzt, dennoch zu einer für die betroffenen Patienten segensreichen Therapie wird Als Voraussetzung — und dies sei an dieser Stelle noch einmal betont — ist der das Leben der Sinneszelle erhaltende Reststoffwechsel notwendig, der bei einer Energiebilanzverbesserung, ungeachtet der weiteren widersprüchlichen Ursachen einer Funktionsminderung zu einem Leistungsanstieg des Hör- und Gleichgewichtsorgans selbst noch Monate nach Störungseintritt führt

Kritisch wäre zu vermerken, daß in unseren Tierexperimenten Bedingungen der Narkose vorlagen, während der Pat nur mit Tranquilizern vorbehandelt die i a ATP-Infusion erhält Die Reflexsituation ist also eine ganz andere Die experimentelle Hypoxämie wirkt auf den gesamten Organismus, während dem akuten Hörsturz ein örtliches Geschehen zugrunde liegt Nicht alle akuten Hörstürze und erst recht nicht alle anderen Innenohrerkrankungen sind gefäßbedingt und Stoffwechselschäden, wie wir schon eingangs erwähnten

Unsere Modellversuche entsprechen nicht den Bedingungen des akuten Hörsturzes, weil ja Hören und Durchblutung bei den Tieren als normal vorausgesetzt werden können Wir bemühen uns außerdem auf biochemischem Wege ATP in der Cochlea nachzuweisen Leider ist uns bisher infolge Mangels einiger Reagenzien der Nachweis des ATP in der Cochlea nach Lowry nicht gelungen So bleiben immer noch Wissenslücken offen, aber der klinische Wert der i a ATP-infusionen mit und ohne O_2-Überdruckzufuhr bei Hörstürzen scheint uns nachgewiesen zu sein

SUMMARY

Intra arterial ATP infusions with glucose and hyaluronidase were successful in 267 patients suffering from various inner ear disturbances but particularly in sudden deafness Good clinical results were noted not only in early treated but also in later cases Recently, we have also used ATP with hyperbaric oxygen, being successful in cases which were previously treated without improvement Investigations in guinea pigs using artificial hypoxia as a model for human sudden deafness, failed because the patterns are not the same So long as an increase of ATP in the cochlear cells cannot be demonstrated after the i a infusions, only an unspecific mechanism can be held responsible for the undoubted clinical successes

RÉSUME

Depuis 6 ans nous employons avec succes intra arterielles infusions de ATP avec glycose et hyaluronidase chez 267 patients pour traitement des maladies d oreille interne. Nous informons de la méthode et de bien resultat comme aussi des constatations en experiences sur l animaux et discutons des aspects patho physiologiques

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DISCUSSION

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A NEW CONCEPT OF VERTEX ERA AND EEG ANALYSIS APPLYING INVERSE FILTERING

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Abstract Evidence for a common anatomical and physiological substrate for the generation of the background EEG and the vertex-evoked response led to the hypothesis that the EEG can be interpreted as the output from a time varying filter driven by (*a*) a noise generator responsible for the background activity and (*b*) a pulse generator creating the evoked response The characteristics of the filter can be estimated by autoregression The application of such a model results in considerable theoretical and practical improvements in the detection of evoked responses Selected averaging on EEGs classified according to the filter characteristics shows improved S/N ratios and considerable diversity between classes Investigations of an estimate of the input signal to the filter derived by deconvolution reveal a sound-evoked potential which is less variable than the response in the EEG The study concludes that the proposed model and ds have a sufficient applicability and physiological relevance to support further developments

In evoked response audiometry (ERA) based on the slow (90–400 msec) components from the vertex, the response detection by means of average computation (Dawson, 1950) and correlation technique (Salomon, 1974) is based on two theoretical assumptions (1) The evoked response (ER) appears in each post stimulus EEG epoch with a constant shape and latency, and (2) the response is independent of the background noise In other words, the bioelectric pick up monitored by a vertex electrode is assumed to consist of a sum of the output from two groups of independent generators, one producing the response, the other the background activity

Serious doubts have been raised as to whether this concept is valid It has been shown that changes in the general level of arousal constitute an important factor influencing the ER The response is enhanced with attention (Davis, 1964) and shows changes in shape and pattern with stimulus correlated tasks (Picton et al 1974, Salomon, 1975) A corresponding systematic change of the EEG with arousal has long been known Thus, both the ER and the EEG may be interpreted as being related to the same factors

More detailed studies show that the average of one group of selected post stimulus EEG intervals with similar amplitude and frequency parameters differs from an average formed from post stimulus intervals selected according to other criteria during the same session (Salomon, 1973, Spreng, 1975) In consequence the average ER appears to be an average of different individual vertex responses being dependent on the degree of attention which changes as a function of restlessness, boredom and anxiety (Davis 1976) This view was further supported through experiments with sequential ERA testing in children (Salomon, 1975) Here it was concluded that the failures of ERA in paedo audiology could be ascribed mainly to the uncontrolled fluctuation of the state of mind (Salomon, 1975)

Evidence suggesting an interdependence between the ER and the background EEG activity has also been established in neurophysiology

Based on a large amount of experimental data it is generally agreed that the EEG is produced by periodic changes of activity in cortical structures These changes are synchronized within zones forming functional entities It has been

shown that the periodicity of these entities is influenced by ascending sensory inputs and that the activity at the same time is related to reverberating ascending and descending signals within closely connected cortical or subcortical structures The EEG recorded from the vertex is a volume conducted sum of activity from the cortex and, although certain areas show preferential frequencies (beta rhythm in the frontal parts and alpha rhythm in parieto-occipital parts), all non-specific areas can show a wide variety in frequency of activity The ER from the vertex in monkeys which is analogous to the human response has been recorded from many sites of the cortex after removal of the scalp and shows the largest amplitude over the precentral gyrus (Nagafuchi & Cody, 1972) In a review (Picton & Hillyard, 1974) it was concluded that the human auditory vertex potential also originates largely from the frontal association cortex This area has no known direct connection to the peripheral auditory system The review suggests that the primary cortex controls the response in the association cortex through cortico-cortical or cortico-thalamo-cortical connections Furthermore, these areas are known to receive impulses from thalamic areas which are activated from sensory inputs irrespective of the body site or sensory modality (Monnier, 1975) Thus, neurophysiological data, besides supporting some degree of common anatomical substrate for the EEG and ER, also suggests overlapping physiological mechanisms

The existence of a common but complex time varying generator system producing the ongoing EEG as well as the ER calls for signal processing methods, which regard the response as dependent on some characteristics of the background activity Considering some aspects of the presented neurophysiological data, it is proposed that the EEG can be regarded as the output of a time varying, though quasistationary filter, driven both by a noise generator responsible for the continuous background activity and by an independent pulse source creating the ER Fig 1 shows a schematic presentation of the hypothesis, implying that the observed EEG background activity $r(t)$ is the convolution of the filter response $h(t)$ and the noise $n(t)$

$$r(t) = n(t) * h(t)$$

Similarly, the ER ($E(t)$) is interpreted as the convolution between an impact pulse $p(t)$ and the same filter response

$$E(t) = p(t) * h(t)$$

The usefulness of the proposal of such a hypothesis depends on several practical and theoretical considerations An evaluation will be attempted based on an analysis of the following four groups of questions and matters

I Does the EEG exhibit stationarity in a sense relevant for a useful and meaningful interpretation of $h(t)$? Do we have feasible methods to estimate $h(t)$?

II By presuming a proper $h(t)$, will it then be a feasible basis for a selective averaging of EEG epochs with effectiveness comparable to earlier studies?

III Inverse filtering of the observed epoch $r(t)$ by the filter response $h(t)$ should produce an input noise estimate that could qualify for the description white noise

IV With a time-locked ER present in the EEG and both potentials being produced from the same generator mechanisms, the input sequence must contain a time-locked event corresponding to the ER Since some variability of the EEG and ER is removed through the inverse filtering with the time varying $h(t)$ it might be expected that the ER related event in the derived input is of a greater constancy than the ER observed This constancy should increase the detectability of a sound-evoked impact on the EEG

I

STATIONARITY

To evaluate the degree of stationarity of the EEG, it is necessary to define the relevant time horizon In this case we are interested in lengths of EEG epochs sufficient to contain a response

as well as adquate information regarding the brain activity On the other hand, the epoch should be so short that it is probable that the results of the analysis are representative for the entire epoch rather than being an average of properties from a number of sub-epochs

An operative criterion indicating a reasonable degree of stationarity in this connection could be to evaluate the "goodness of fit" by means of the relative magnitude and conformity of that part of the EEG which is not explained by the filter, e g the 'noise" part $n(t)$ according to the model in Fig 1

The EEG signal was low pass filtered at 22 Hz (6 dB/octave) and sampled every 16 msec for a period of 2 4 sec (150 points) The analysis was performed on the first 128 sample points This number was regarded as sufficient for the production of reliable statistics and practical for computer handling A 300 msec prestimulatory recording ensured a central position of a possible response in the recorded epoch

By applying this epoch length it is observed that the method of analysis produces filter characteristics of significant dispersion between succeeding epochs This is taken as an indication at the chosen epoch length is not too long

Fenwick et al (1971) and Wennberg & Zetterberg (1970) in contrast recommend a duration of 20 sec Our experience indicates, however, that both background activity and single response shapes may change significantly within periods of a few seconds

Stationarity of epoch lengths of 2 sec implies that the spectrum of the EEG is limited downwards at 0 5 Hz However, in this study components of lower frequency are regarded as insignificant to the brain processes under investigation Consequently, action is taken to reduce the influence of these components on the estimated filter characteristics by demeaning and detrending (Bogert, 1972) the observed EEG epoch before any analysis begins

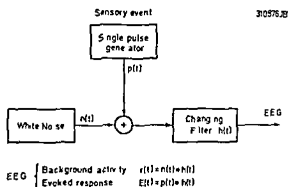


Fig 1 Model applied for analysis of the EEG

Method of Analysis

Several methods of differing complexity exist for the description of the properties of a stationary time sequence The easy access to computational power in recent years has concentrated the interest on rather complex models requiring iterative estimation procedures

All models considered are based on a filter hypothesis, as shown in Fig 1, but apply sampled data for numerical reasons and will consequently be presented in discrete form In the following the sequence of sampled filter input values is denoted n_t, and the sequence of output sample values representing the observed EEG is denoted r_t Three models are considered

The Moving Average model makes an estimate of the output sequence r_t on the basis of a weighted average of the input sequence n_t according to the formula

$$\hat{r}_t = n_t + b_1 n_{t-1} + b_2 n_{t-2} \dots b_q n_{t-q}$$

This model requires knowledge of the inpu sequence n_t or an estimate hereof performe during the analysis

The Autoregressive model estimates the ou put sequence on the basis of the previous outpu values

$$r_t = a_1 r_{t-1} + a_2 r_{t-2} \dots a_p r_{t-p} \quad (1$$

A third model combines the schemes of th other two

$$r_t \quad a_1 r_{t-1} + a_2 r_{t-2} \dots a_p r_{t-p} + n_t + b_1 n_{t-1} \dots b_q n_{t-q}$$

The complexity of the models is determine by the orders p and q, often chosen between and 15 All parameter sets (b s and a s) are usu

ally estimated using the method of least squares —that is Determine a's and/or b's in order to minimize

$$\sum_{i=1}^{N}(r_i-\hat{r}_i)^2$$

presuming n_i's are equally distributed and independent N is the number of sample values in the time sequence under analysis

Zetterberg (1969) and Wennberg & Zetterberg (1970) applied the combined model in their large sample analysis of EEG concerned with the detection of epileptic spikes Fenwick et al (1971) applied the autoregressive scheme (1) in their study Since we have a particular in terest in the (hypothetical) input sequence to the presumed filter and since our analysis is intended to be performed repeatedly within a test, we have made the autoregressive model our first choice *The autoregressive model does not* anticipate an evaluation of the input sequence and offers reasonable computational simplicity

The implications and algorithms of an auto regressive model are treated excellently in detail by Atal & Hanauer (1971) in a paper concern ing speech analysis and synthesis and will only be treated here in a few aspects

When excited by a series of independent samples from a white noise source the hypothesized filter creates an output with approximately the same statistical properties (covariance matrix) as the EEG epoch analysed The characteristics of this filter are easily obtained from the autoregressive coefficients (a_j) The output of the filter is assumed to be zero until a certain instant where it reaches the value one Then, by letting the autoregressive scheme be repeatedly applied to the output sequence thus initialized, the desired filter impulse response $h(t)$ will be produced as an output sequence

The derived impulse response may not always converge towards zero When a_j's are estimated from the *autocorrelation function*, however, convergence is automatically ensured (Atal & Hanauer, 1971)

The resulting filter is an all pole filter, i e the method is unable to estimate possible zeros in the transfer function This is a logical consequence of the scheme, since results are estimated from the *output* of the filter

Characteristics such as resonant frequencies and related bandwidths may easily be estimated from the autoregressive coefficients (see, e g Atal & Hanauer, 1971)

The complexity of the model is determined by the order p applied and varies from epoch to epoch The actual value of p is determined during analysis One consideration is that p is chosen so as to be as small as possible while creating a filter of sufficient complexity to explain most of the variance cf the data Another contributor to this choice is the number of successive autoregressive coefficients largely different from zero Accurate information on the adaptive determination of p is given by Hansen (1974) Values of p between 2 and 9 were used in this study

II

CLUSTERING METHOD

The purpose of clustering (Sokal, 1974) is to select EEG epochs containing similar evoked responses According to the hypothesis stated this is equivalent to a classification of EEG epochs with respect to their properties The estimated filter impulse response, described above, establishes a basis for an evaluation of these properties

The impulse responses are analysed with respect to a rather arbitrary choice of the three simple attributes Curve lengths, mean value and number of peaks and valleys The clustering procedure is based on the Minimal Spanning Tree (MST) proposed by Zahn (1971) in the following way

Each impulse response is characterized by three values obtained from the attributes by multiplication with chosen weighting factors In the three-dimensional space, these values are used as coordinates for a point representing a single EEG epoch The points representing all EEG epochs are interconnected with straight lines, branches, forming a tree-like structure.

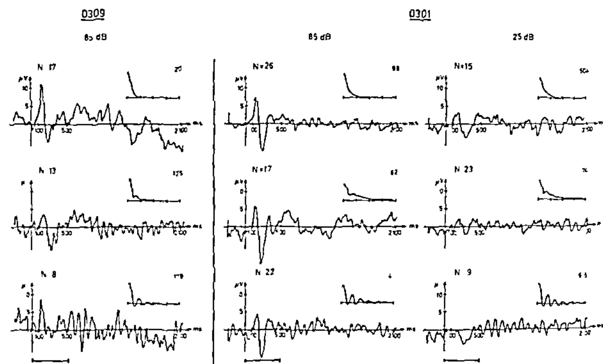


Fig 2 Selected averages Averages of EEG epochs selected according to similarities of the corresponding impulse responses The impulse responses are exemplified in insets The stimulus durations of 0 5 sec are indicated at the bottom *Ordinate* Mastoid vertex voltage *Inserts* Time scale 160 msec/division *Ordinate* Arbitrary voltage units Data from subject 309 at 85 dB SL and subject 301 at 85 and 25 dB SL, 1 000 Hz

This connection is created in a way which minimizes the sum of the length of the branches, thus creating the MST tree The division of the points into clusters is based on an evaluation of the distances between the points The length of any branch in the MST tree is related to the average of the length of neighbouring branches and their neighbour branches If this ratio exceeds a chosen critical value (e g 2 0) the branch is considered as connecting points in two different clusters Removal of such branches divides the tree into subtrees (clusters) Consequently, the choice of the critical value will influence the number of clusters With appropriate weighting of the attribute qualities and a reasonable choice of the critical value 4 to 5 main clusters with good separation may be obtained Detailed description of the implementation of these algorithms is given by Hansen (1974)

Results and Discussion

In 6 adults and 3 children the average ER was produced from clusters of selected post stimulus EEG epochs These selected ER showed a variety of patterns in response to constant stimuli in all subjects even when the EEG was recorded within a few minutes Small changes in the impulse response were often accompanied by large ER changes Fig 2 shows examples from two adults, one at two intensities In the latter, as in other subjects, certain systematic but individual trends in the ER appear with changes of the cluster (i e the impulse reponse) irrespective of intensity This trend does not seem to be consistent between subjects

It has been suggested that the ER is composed of a series of successive components which could selectively be enhanced in an orderly way by systematism of experimental conditions (Davis

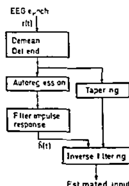


Fig 3 The procedure applied in deriving the input sequence

1976) This would suggest that the observed fluctuations between the patterns of the selective ER is caused by a varying contribution of the suggested components Such a hypothesis would imply that general rules regarding the ER pattern exist We found, as mentioned, no evidence of such systematism, even though the experimental conditions were framed to enhance α and β activity (see IV)

The observed definition of ER patterns within each cluster can be regarded as biological evidence for the relevance of the established $h(t)$ filter characteristics At the same time, this improved definition anticipates a higher S/N ratio which is of importance in audiological as well as in physiological studies

III

INVERSE FILTERING

Inverse filtering of the EEG epoch $r(t)$ with the estimated filter impulse response $h(t)$ is equivalent to determining the input signal $n(t)$ to the filter This signal must satisfy the convolution formula $r(t)=h(t)*n(t)$ applied in its discrete version

$$r_i=\sum_{j=0}^{\infty} h_j \; n_{i-j} \qquad (2)$$

In practice, the sum is extended only to values where h_j is significantly different from zero

The inverse of this formula may be established and applied in the estimate of the n_i's (Valentinuzzi & Volachec, 1975) This discrete deconvolution method, however, is susceptible to large errors, since it involves chain multiplications Another approach is to apply the correspondence between convolution in the time domain and multiplication in the frequency domain

Let $F(y)$ denote the discrete Fourier Transform of the sequence y, Then (2) is in informal notation equivalent to

$$F(r)=F(h)\; F(n)$$

By rearrangement

$$F(n)=\frac{F(r)}{F(h)}$$

hence

$$n=F^{-1}\left(\frac{F(r)}{F(h)}\right)$$

where F^{-1} denotes the inverse Fourier Transform The crucial point in the feasibility of this method lies in the division of (complex) spectral values However, recalling that the autoregressive scheme only generates all pole filters, the Fourier Transform $F(h)$ of the filter impulse response cannot be zero and, consequently, division can always be performed Some effects of the implicit assumption of periodicity inherent in the discrete Fourier Transform may be observed These effects are related to a possible discontinuity at the epoch limits and may appear at the initial and final value of the estimated noise sequence The effects may be suppressed by applying cosine tapering for e g 10 points at the beginning and end of the observed epoch (Bogert, 1972)

The signal processing applied to the observed EEG epoch to obtain the derived input is schematically indicated in Fig 3

Provided suitable methods of analysis exist, the main hypothesis implies that the input sequence is indistinguishable from white noise with respect to the statistical properties of the sequence Inspection of the autocorrelation functions of the input sequences revealed that these

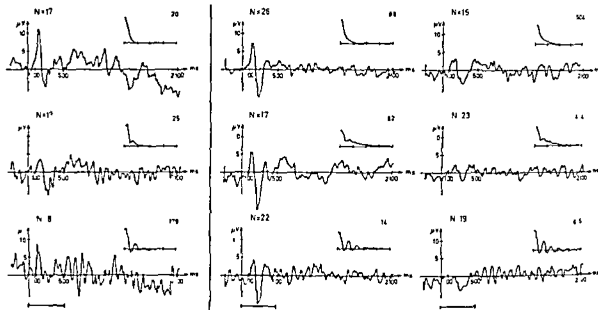


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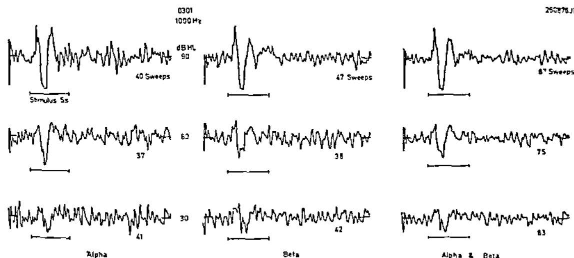


Fig 5 Averages of derived inputs Recordings made during induced α activity (*left*) and β activity (*middle*) A summation of the α and the β averages is shown (*right*) Time scale and ordinate, see Fig 4 Data from subject 301

lus level A similar test and analysis was performed in 3 children age 6–10 months, but in these tests no control of the brain activity was attempted and in fact all 3 were very restless and periodically agitated and crying during the procedure The stimulus levels were determined relative to thresholds estimated from clinical findings and permitting 5 dB adjustment It turned out later on that one child suffered from a moderate hearing loss

Results

In all supra threshold stimulus recordings, averages of $n(t)$ in the adults produced a distinct input potential, denoted IP (corresponding to $p(t)$ in Fig 1), both in the $n(t)$ obtained during the "α" and "β" procedure This IP potential although showing individual differences displayed almost identical patterns during the two modes (see Figs 4 & 5) Thus, in contrast to the normally used averaged EEG response, the IP does not in any way display the variance of pattern illustrated in Fig 2 The conformity of the patterns permit pooling of the "α" and "β" IP's, as shown in Fig 5 This indicates that the signal detection of the IP is independent of the peristimulatory behaviour Whereas the production of the IP could be the result of the effectiveness of the mathematical procedures (separation between systematism and unsystematism), the constancy of the IP is a biological finding of great promise

In the children the IP was easily recognized at the two higher intensities (Fig 6) This was in contrast to the normal average EEG where a response rarely is more prominent than the random activity of the average background noise In both adults and in children the characteristic feature of the IP potential appeared not only by virtue of the response amplitude but also particularly as a result of the contrast between the IP and the uniform background noise

Discussion

Acoustically elicited changes in the EEG are used in audiology as an indicator of sound perception Although knowledge of origin, pattern and physiology of these changes is of the greatest interest, the audiologist is primarily concerned with the ability to give a binary answer to the question Is a stimulus-related signal present in the EEG? The hypothesis presented has been shown to permit introduction of adequate signal detection processes not yet in use

The present hypothesis does not pretend to give a faithful functional picture of the brain

010976JB2

1706 (infant 10 month)
1kHz

HL

90dB n=57

60dB n=54

30dB n 60

stimulus 5s

Average derived input 15 Hz Low pass

Fig 6 Average derived input from a 10-month old child at 3 intensities The averages digitally low pass filtered at 15 Hz

mechanisms involved The generation of the ER is very complex, involving many cerebral mechanisms of which only few are known On the other hand, the mechanism described in the introduction could suggest a simple generator model which is in accordance with our proposed model of analysis (see Fig 1)

The EEG is interpreted as the weighted sum of activity from a time varying multilevel feedback system excited by uncorrelated events responsible for the generation of the background activity (Fig 7)

The varying characteristics of the EEG are interpreted as the result of varying gain of the branches sometimes emphasizing oscillations at one frequency and sometimes at another, dependent on the functional state of the system

The contribution to the voltage at the summing point from each individual random source can be represented by a transfer function associated with the source's attack point Provided the attack points are within the feedback loops it can be shown that the transfer functions hav identical pole configurations These poles, bein

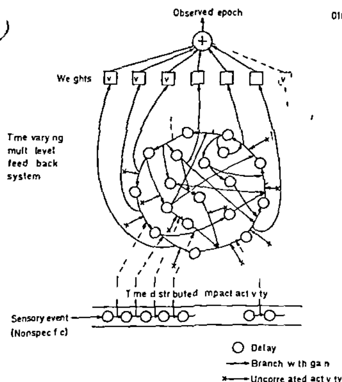


Fig 7 Generator model for the ongoing EEG and evoked response

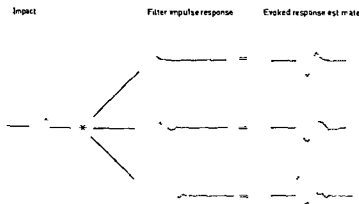


Fig 8 Three examples of evoked response estimates determined by convolving an estimate of the derived input potential (impact pulse, IP) with experimentally obtained filter impulse response

of great importance for the characterization of the system, corresponds to the poles of the filter estimated by the autoregressive scheme

The ER is assumed to appear as a consequence of a (time distributed) impact on the multilevel feedback system generated by a non specific subcortical structure

Such a model would be able to account for the inhibition between modalities and by introduction of saturation phenomena, the tendency of synchronization of the EEG rhythmic stimulation Furthermore, the model permits large ER latencies involving a large number of synapses On the basis of frequency analysis of the EEG epoch with and without stimulus, Sayers et al (1974) have observed a remarkable synchronization of phase of low frequency components in response to stimuli without accompanying change in spectral power The present model is also able to simulate this finding, provided that saturation phenomena and refactory periods are incorporated

Signal detection, according to the hypothesis presented, can be used in clinical audiometry in different ways

(*a*) An improvement of the S/N ratio of the classical averaged EEG response can be obtained using a selective average as outlined This principle could also enhance neurophysiological studies.

(*b*) The average ER related potential, IP, in the derived input can be used as an indicator of perception in the same ways as the standard EEG response Besides visual identification, the IP potential seems extremely well suited for automatic evaluation

(*c*) The constancy of the IP potential can be used to draw an individual noise free template This template can be used to estimate the ER from a certain post stimulus epoch by convolution with the corresponding $h(t)$ from this very epoch Fig 8 shows three synthesized responses ($h(t)$'s and the template created from data from subject 0301) It is noteworthy how these estimated responses correspond to the variance of shape observed in the selected average (Fig 2) After having created the pattern of a possible signal hidden in the EEG, a series of well known signal detection procedures and statistical methods could be applied in order to establish a confident answer to the audiologist's question

Further studies will be concerned with an evaluation of the different approaches

ACKNOWLEDGEMENT

In co-operation with our department, Soren Skogstad Nielsen, M Sc. has been an inspiring advisor for the thesis project (Hansen 1974) referenced. He has also been responsible for the computer implementation of Hansen s results at the Department of Data Processing in Medicine Gentofte University Hospital

RÉSUMÉ

L'epoque temporelle d'électro-encéphalogramme $r(t)$ est interpretée comme bruit blanc $n(t)$ filtre par un filtre $h(t)$ qui s'adapte et varie avec le temps $(r(t) = n(t) * h(t))$ Les caractéristiques du filtre sont estimées en appliquant une séquence autoregressive dérivée de l'encephalogramme Des expériences cliniques montrent que l'excitation dérivée par la déconvolution de l'encéphalogramme ressemble de près au bruit blanc. En outre, l'existence d une réponse evoquée dans l'encéphalogramme se reflete dans l'excitation derivée De meme, des découvertes cliniques démontrent que les réponses trouvees dans l'excitation dérivée sont plus indépendant de l'age et de l'activité que les réponses dans l encéphalogramme L'applicabilité de la hypotése proposée est discuté

ZUSAMMENFASSUNG

Das EEG $r(t)$ wird als das Resultat der Filtrierung eines weißen Rauschens $n(t)$ mit einem adaptiven zeitvariierenden Filter $h(t)$ ausgelegt $(r(t) = n(t) * h(t))$ Die Charakteristika des Filters werden mittels einer autoregressiven Serie geschätzt Klinische Beobachtungen zeigen, daß die hypothetischen Eingangssignale zum Filter, bei inverser Filtrierung des EEGs hergeleitet, weißes Rauschen gut approximieren Auch ist ein Response in das EEG im hergeleiteten Eingangssignale wiederzufinden Dieser hergeleitete Response erscheint weniger abhängig von Alter und Aktivitat als der Response im EEG Die Anwendbarkeit der Hypothese wird von verschiedenen Seiten erleuchtet

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DISCUSSION

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MACROSCOPIC AND ULTRASTRUCTURAL FINDINGS IN SOME DISEASES OF THE FACIAL NERVE

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Abstract The authors describe the findings observed in 10 cases of Bell s palsy, 1 case of traumatic facial paralysis 1 case of congenital facial paralysis and 1 case of Melkersson Rosenthal syndrome The chorda tympani nerve of these patients was studied employing the electron microscope comparing it with that of 5 patients having otosclerosis Our ultrastructural findings appear to confirm that the chorda tympani nerve presents a similar degenerative behaviour in those diseases studied by us The degenerative stages range from a lesser to greater degree in the following order Bell s palsy traumatic facial paralysis congenital facial paralysis Melkersson Rosenthal syndrome

Peripheral facial paralysis is an interesting subject, presenting numerous pathological enigma which require elucidation The fundamental problem is understanding its clinical pathological evolution In other words, can the nerve degenerate, and if so, what would be the most suitable treatment in each case

In idiopathic facial paralysis, Minkowski (1891) and Dejerine & Theohari (1897) described swollen Schwann sheaths in the distal fallopian segment of the facial nerve in man Since then these findings have been confirmed by optical (Jongkees 1954 Kettel, 1959 Miehlke, 1960, McGovern, 1970 and others) and electron microscope studies (Blatt & Freeman, 1966–68, May & Schlaepfer, 1975, Karatay et al, 1976)

Jongkees (1954) and Sadé et al (1965) found a normal epineurium in patients having Bell s palsy Fisch & Esslen (1972) have observed edema, red swelling and marked vascular injection in the meatal and labyrinthine segment of the facial nerve

Furthermore, numerous laboratory experiments have been reported, where attempts have been made to injure the facial nerve, the object being to verify the degenerative evolution and the effects of surgical decompression The principal ones are (1) those that attempt to produce a mechanical lesion (Sullivan & Smith, 1950, McGovern & Hensel, 1961, Jain & Sharma, 1964 Devriese, 1972), and (2) lesions induced by heat, cold, virus or by chemical substances (Sullivan & Smith, 1950, Coassolo, 1952, McGovern et al, 1966–72, Boyle, 1966, 67, 72, Kumagami, 1972)

In the present work we intended to study macroscopically and ultrastructurally several facial nerves affected by diverse etiological agents

MATERIAL AND METHODS

Our studies were based upon five groups of patients The first or control group comprised 5 otosclerotic patients having no history of facial paralysis Their ages ranged from 20 to 45 years Chorda tympani nerve specimens were obtained while performing stapedectomy All patients were preoperatively tested for chorda tympani function, employing electrogustometry and measuring submandibular salivary flow

The second group was formed by 10 patients (7 males, 3 females) having Bell s palsy Their ages ranged between 6 and 50 years The patient s initial symptomatology was ear ache associated with facial numbness in the trigeminal and cervical ipsilateral areas Between 4 hours and 3 days later the facial paralysis set in, usually

initiated by a deviation of the mouth and later the patients claimed that they could not close one eye Some noticed a loss of taste and vertigo coinciding with the facial paralysis One female patient showed a normal electrogustometry and electromiographic response and a salivary flow between 25-50 % of normal, so that a neurectomy of the chorda tympani was performed In the remaining 9 patients the electro gustometry response and the stapedial reflex test were depressed or absent, the submandibular salivary flow being 25 % below normal, the Schirmer tear test normal, and the electromiographic response demonstrating complete de nervation The glucose serum level was normal in all patients A transmastoid decompression from the stylomastoid foramen to the geniculate ganglion was performed Electron microscopic studies of the chorda tympani nerve were carried out in 5 patients The interval between the onset of paralysis and the operation was between 20 and 50 days

The third 'group' comprised a 6-year old patient with a facial paralysis due to head trauma After the injury the child presented only bleeding from the ear, but 5 hours later the cial paralysis set in The clinical tests de- onstrated a denervation beneath the geniculate ganglion and 48 hours later a surgical decom pression was performed

The fourth group is an infant patient of 18 months having a congenital facial paralysis Pregnancy and delivery were normal Observed in the electromiographic test was a complete denervation pattern of the frontal muscle and a partial one of the zygomatic and orbicularis oris The Schirmer tear test was normal A decompression was promptly carried out

In the fifth group there was a female patient suffering Melkersson Rosenthal syndrome with a history of recurrent facial paralysis, facial swelling, ear ache and lingua plicata All clinical tests demonstrated the presence of a denervation beneath the geniculate ganglion for which a decompression of the tympanic and mastoid segment of the facial nerve was performed 30 days after the onset of paralysis

During surgery the specimens were immedi ately fixed in a 2 % solution of glutaraldehyde in phosphate buffer at pH 7 4 Details of our technique have been published in previous papers (*Marco et al*, *1971*, *Sanchez Fernández* et al , 1972) The samples were examined with a Philips EM 300 electron microscope at 60 kV Photographs were taken on 23 d 50 Gevaert Scientia films

FINDINGS

Macroscopic pathology

In Bell's palsy the epineural nerve sheath generally appears to be very thick The mastoid and sometimes the tympanic portion of the nerve presents edema and the nerve appears strangulated within its sheath, protruding after opening of the nerve sheath Occasionally we have observed a circumscribed ballooning of the nerve sheath Upon opening the sheath a protrusion was produced by a hemorrhagic infarct within the nerve, situated just below the pyram idal turn We observed in this case, at the end of the operation, an abrupt ending of the bulging in contrast to the normal tympanic portion of the nerve The chorda tympani nerve always appears edematous and reddened

In traumatic facial paralysis the nerve was integral However, edema was present in the tympanic and mastoid segment, plus numerous *intrafascicular hemorrhages*

In the congenital facial paralysis there was a *large hematoma situated within the* nerve sheath at the tympanic portion

In the Melkersson Rosenthal syndrome a hemorrhagic infarction was observed below the pyramidal turn and edema of the mastoid por tion of the nerve

ULTRASTRUCTURAL FINDINGS

Chorda tympani from otosclerotic patients

The chorda is formed by myelinated and un myelinated fibres whose proportions vary be tween one fourth to one tenth respectively (Fig *1 a, b*) In some specimens we observed a severe splitting in an area of the myelin sheath, while

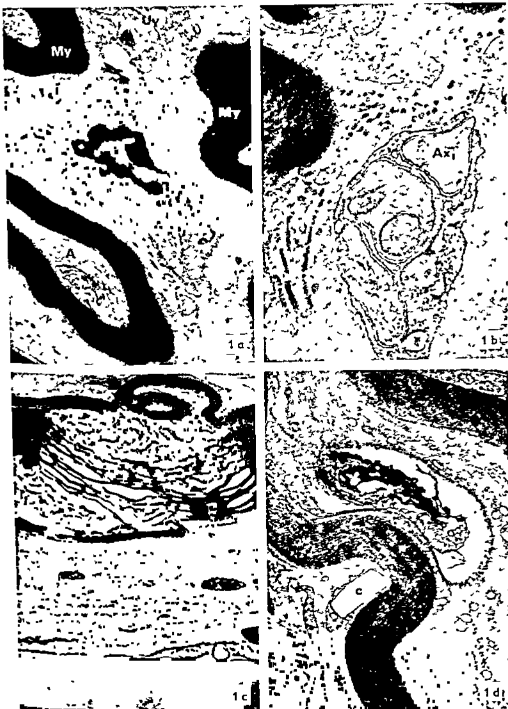


Fig 1a Chorda tympani from an otosclerotic patient (case 1) Myelinated fibre (*My*) unmyelinated fibre (*Uy*) axon (*A*) collagen fibres (*Co*) × 10 200

Fig 1b Chorda tympani from an otosclerotic patient (case 3) The axon cylinder (*AxI*) is enveloped in an area only by the basement membrane which separates it from the extracellular space (*arrow*) × 32 000

Fig 1c Chorda tympani from an otosclerotic patient (case 4) Note the severe splitting on one side of the myelin sheath, in contrast to the other which retains a normal ultrastructural pattern Mitochondria, neurotubules and neurofilaments in the axon are normal in appearance × 18 000

Fig 1d Bell's palsy Note the normal ultrastructural pattern of the myelin sheath. In the Schwann cell cytoplasm appears a crystalline inclusion (*c*) See text for further details × 19 000

the axon remained normal in appearance, as also did the other side of the same sheath (Fig 1*c*)

Bell's palsy

Within the cytoplasm of myelinated Schwann cells one can observe crystalline bodies and lipid droplets Some axons presented an appearance of vacuolar degeneration The vacuole containing a myelin figure probably originated with the splitting of the innermost lamellae of the myelin sheath (Fig 1*d*)

The total content of unmyelinated fibres appears increased in proportion to the myelinated ones Their cytoplasm presents a foamy appearance that may be due to lipid inclusions (Fig 2*a*)

In the Schwann cell cytoplasm of some unmyelinated fibres, an inclusion material appeared, arranged in alternating bands of electron dense and electron lucent substance plus some spheres of a homogeneous substance These findings are similar to the "zebra bodies" (Fig 2*b*) At times the cytoplasm of demyelinated Schwann cells exhibits areas of a vacuolar [illegible] and others that contain multiple [illegible]c lamellar formations, myelinated in appearance, that probably correspond to autophagic vacuoles, which finding expresses the distinct phases of myelinated fibre digestion (Fig 2*c*)

Traumatic facial paralysis

It is possible to find some normal fibres, while others give the appearance of Wallerian degeneration in various evolutional stages According to the severity of the lesions, they can be described as follows

Stage A Vacuolar degeneration of the axon with disappearance of mitochondria neurotubules and neurofilaments The myelin sheath is conserved and one can observe a normal morphology of some of the neighboring axons (Fig 3*a*)

Stage B Hydropic degeneration of the axon and cytoplasm in myelinated and unmyelinated Schwann cells (Fig 3*b*)

Stage C Splitting and disintegration of the innermost lamellae of the myelin sheath At times one can see a complete interruption of all the lamellae of the myelin sheath remaining Schwann cell basal membrane (Fig 3*c*)

Stage D Accumulation of myelin figures in the Schwann cell cytoplasm (Fig 3*d*)

Stage E Formation of multiple concentrical myelin figures (ovoids) and fusion areas of myelinated material alongside others in a state of granular degradation in the Schwann cell cytoplasm Some of these myelin ovoids retain their normal lamellar structure, while others have a homogeneous appearance (Fig 4*a*)

Congenital facial paralysis

Schwann myelinated and unmyelinated cells give an appearance of vacuolar degeneration of the cytoplasm Within the vacuoles, degenerative mylin figures can appear (Fig 4*b*)

In the axon it is possible to find edema, vacuolar degeneration and an accumulation of lipid like droplets (Fig 4*c*)

The myelinated fibres disorganize and a dense granular substance appears in proximity to large areas of myelin like material, around which numerous collagen fibres can be seen (Fig 4*d*)

Melkersson Rosenthal syndrome

There were very few normal myelinated fibres and we have not seen any unmyelinated ones at all In areas where myelinated fibres appeared these showed vacuolar degeneration of their axons and a profound disorganization in the myelin sheath Most parts of the specimen appear to be occupied by a granular material

Fig 2a Bell s palsy Schwann cells from demyelinated nerve fibres are foamy in appearance In some of the vacuoles one may observe myelin figure debris while others appear empty or contain a few dots × 12 000
Fig 2b Bell s palsy In the Schwann cell situated in the centre of the photograph one can observe a zebra body and lipid inclusions See text for details × 32 500
Fig 2c Bell s palsy Schwann cell cytoplasm (*Sc*) See text for description Abbreviations as in previous figures 10 000



Fig 2d Bell's palsy Some Schwann cells contain multiple concentric myelin lamellae and vacuolar cavities resembling lipid droplets, which at times appear free in the intercellular space The myelinated Schwann cell cytoplasm contains a homogeneous substance of regular electron density plus other degenerated myelin lamellae Observe the splitting of the innermost lamellae of the myelin sheath. × 9 200









Fig 3a Traumatic facial paralysis Stage "A" Vacuolar axon (*V*) See text × 17 000

Fig 3b Traumatic facial paralysis Stage "B" Hydropic degeneration (*Hy*) See text for details × 24 000

Fig 3c Traumatic facial paralysis Stage "C" Interruption of myelin sheath × 40 000

Fig 3*d* Traumatic facial paralysis Stage "D" × 13 500





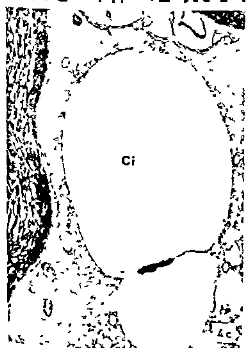




Fig 4a Traumatic facial paralysis Stage 'E' Ovoids (*Ov*) granular degradation (*Gr*) 15 000
Fig 4b Congenital facial paralysis Vacuolar degeneration (*Va*) See text 18 500

Fig 4c Congenital facial paralysis Lipid like droplets (*Ci*) × 15 000
Fig 4d Congenital facial paralysis Collagen fibres (*Co*), degenerative myelin debris (*Dm*) 18 000

of great electron density that probably results from degradation of the myelin sheath An amorphous material of low electron density can also be observed there Immersed in this material there are cellular processes and debris, some erythrocytes and numerous collagen fibres

DISCUSSION

Macroscopic findings Surgical observations

In Bell's palsy our macroscopic findings are in agreement with those of Ballance & Duel (1932), Kettel (1959), Miehlke (1960–73), Cawthorne (1965), Jongkees (1968–72) and many others who have reported swelling in the mastoid portion of the nerve Nevertheless we have also observed in many cases a swelling in the mastoid and tympanic portion of the facial nerve Pulec (1974) described how the distribution of edema in 100 cases of Bell's palsy was 40 % in the mastoid segment, 40 % in the tympanic portion, 5 % in the geniculate ganglion area and 15 % in the internal auditory canal

Fowler Jr (1963) and Cawthorne (1965) found hemorrhagic infarctions on both sides of the stylomastoid foramen and in the internal [illegible]stic meatus Boyle (1967), in experimental [illegible]l nerve paralysis produced in monkeys by an ultrasonic beam, observed a hemorrhagic area in proximity to the stylomastoid foramen In some cases of Bell's palsy we have noted an area of hemorrhagic infarct situated just below the pyramidal turn in the mastoid portion of the nerve This same finding has been observed by us in the Melkersson Rosenthal syndrome

Blatt & Freeman (1966) and May & Schlaepfer (1975) observed the swelling and reddish appearance of the chorda tympani nerve This finding coincides with our observations

In traumatic facial paralysis we have found intraneural hemorrhage and edema, while nerve continuity was maintained

Our patient with congential facial paralysis presented a circumscribed hematoma in the tympanic segment of the facial nerve Such bleeding probably is due to mechanical damage during delivery This patient recovered all his motor functions 6 months after surgical decompression

Ultrastructural findings

May & Schlaepfer (1975) demonstrated that chorda tympani is composed of myelinated and unmyelinated fibres in a ratio of 10 1 Our findings are in agreement with these authors

The uncommon myelin sheath alterations that appear in some of our control specimens may be interpreted as resulting from the surgical trauma occasioned by stretching the nerve during its extraction This is a common finding observed in the peripheral nerves (Sunderland, 1968) In this way, Boyle (1967) noticed in the monkey, a very small area of demyelinization 3 weeks after decompression of a normal facial nerve, and Binns (1968) observed the degeneration of cat facial nerve, when it is lifted out of the fallopian canal and then replaced in its original bed

The crystalline inclusions and "zebra bodies found by us in the Schwann cells' cytoplasm of some myelinated and unmyelinated nerve fibres in Bell's palsy are, in our opinion unspecific changes They may represent a reaction of these cells to various injuries In support of this hypothesis, Sun & White (1974) observed crystalline inclusions in peripheral nerves of the rat after alcohol injury, and Bischoff (1970) described "Zebra bodies' in cases of gargoylism, interstitial neuropathy and diabetic neuropathy

Other usual findings in the unmyelinated Schwann cell cytoplasm are the lipid inclusions and numerous autophagic vacuoles that can be found over the entire section of the nerve Both findings may be the result of myelin sheath and axon digestion The fact that Schwann cells turn themselves into macrophages in tissue cultures of embryonic and adult rat nerves was reported by Weiss & Wang (1945) Moreover numerous authors (Luse, 1965, Carvioto, 1967) share the opinion that *in vivo* the Schwann cells remove their myelin debris by autodigestion when a degenerative process affects the nerve On the other hand, Raine et al (1971) observed in experimental allergic encephalomyelitis that

in the process of demyelination the macrophages participate actively Also Liu (1974) demonstrated that the degenerated products of myelin, axon and Schwann cells were removed by macrophages originating from blood monocytes and vascular pericytes

Blatt & Freeman (1966) proposed that the pathogenetic mechanism of Bell's palsy is an initial inflammatory lesion of the chorda tympani nerve and later, by a retrograde extension of the inflammatory process, the facial nerve trunk is altered We cannot support their hypothesis however, because the absence of inflammatory cells and virus like particles was a constant finding in our cases

May & Schlaepfer (1975) attempted to prove that chorda tympani nerve was involved before the facial nerve, in order to confirm Blatt & Freeman's hypothesis They correlated the earlier clinical involvement of chorda tympani function with the electron microscopic findings observed in group B of their patients In their electron micrographs, the acute axonal degeneration or loss is probably similar to hydropic axonal alteration as seen by us in traumatic facial paralysis Also, their myelin degradative debris is structurally very similar to our multiple, concentrically myelinated figures We believe that these structural changes may represent the initial stages of Wallerian degeneration secondary to the edema and intrafascicular hemorrhage present in the tympanic and mastoid portion of the traumatized facial nerve and also to a hematoma found in the tympanic portion in a patient having congenital facial paralysis

We believe that clinical symptoms of chorda tympani injury will appear early when the initial alteration is located in this nerve, though in our patients the loss of taste coincided with the onset of facial paralysis However, May & Schlaepfer (1975) have described how taste was altered in 4 of 7 patients prior to the onset of facial paralysis

Blatt & Freeman and May & Schlaepfer (1975) share the opinion that in Bell's palsy the chorda tympani nerve and the main trunk of the facial nerve may present a similar pathological behaviour However, Saito et al (1970) described a selective degeneration of motor fibres in a patient with facial paralysis due to an astrocytoma of the brain stem In a second patient, suffering from surgical injury to the geniculate ganglion, Saito describes a selective degeneration of the sensory components, while the motor ones remained normal On the other hand, Schuknecht (1974), studying a case of incomplete recovery from Bell's palsy, reported partial loss of motor fibres, yet having a normal sensory bundle

Beaver et al (1965), in patients with trigeminal neuralgia, described vacuolization of the ganglion cell cytoplasm in the Gasserian ganglia Also, there was a proliferation of myelin sheaths, with formation of whorl like bodies (ovoids) Occasionally in some myelinated and unmyelinated fibres the axon is separated from the interstitial tissue only by the basement membrane Kerr & Miller (1966) confirm these findings of Beaver et al and added some additional facts about the axon's degeneration They noted the absence of inflammatory infiltrates and viral inclusion bodies Also, in glossopharyngeal neuralgia, Ishii (1976) observed, in the resected IX nerve, a disorganization of the myelin sheaths and vacuolization of the extruded axon Kumagami (1972) produced a facial nerve paralysis by inoculating rabbits with herpes simplex virus through the stylomastoid foramen Electronmicroscopic study of one animal 13 days after inoculation revealed degeneration of Schwann cells, myelin sheaths and vacuolization into the axon In another animal which survived for 223 days with severe paralysis the nerve was replaced by collagen fibres

The changes described by these authors in such varied pathological conditions are similar to those found in our material Consequently, we may speculate that none of them is specific

In traumatic facial paralysis we have found a degenerative Wallerian pattern, which spans diverse stages from "A" through "E" "A" represents the initial alteration and "E" the most advanced phase It is important to point

out that in the same sample of histological tissue one may observe all these stages and also the presence of apparently normal fibres

Usually, three stages are distinguished in Wallerian degeneration The first is characterized by axonal degeneration and myelin fragmentation These findings correspond to stages "A" through "C In the second there is a chemical destruction of myelin as is clearly observed in stages "D-E" This patient was studied 48 hours after the onset of the paralysis As such, we can follow up the initial ultrastructural findings in such paralysis The third Wallerian stage corresponds to the formation of neurilemmal tubes and increased collagen content of the nerve This latter finding is clearly observed in the congenital facial paralysis and in the Melkersson Rosenthal syndrome specimens

In the Melkersson Rosenthal syndrome the motor recuperation was complete 5 months after the surgical decompression and 40 days in the case of traumatic facial paralysis However, the recuperation incidence in our Bell s palsy cases was only 80 % at 6 months, as described by Pulec (1974) and others

In our Bell s palsy patients the large increase in ,yelinated fibres in the chorda tympani nerve d be an expression of neural regeneration within the Wallerian degeneration phenomenon

It is possible that the discrepancy between important histological alterations of the chorda tympani nerve and the rapid recuperation of the motor fibres may be attributable to the distinct degenerative behaviour of both nerve fibres, as pointed out by Saito et al (1970) and Schuknecht (1974) We may speculate that the chorda tympani and the facial nerve can present both segmental demyelination and myelin regeneration phenomenon, as described by Webster (1964) and others in the peripheral nerves According to this hypothesis the motor fibres may regenerate faster than the sensitive ones Schuknecht (1974) described a facial nerve that appears normal on histological study following recovery from Bell's palsy

We have not found any evidence of diabetes in any of our patients, even though Korczyn (1971) pointed to an incidence of 66 % in Bell s palsy

Our ultrastructural findings seem to confirm that the chorda tympani nerve presents a similar degenerative behaviour in those diseases studied by us The degenerative stages range from a lesser to a greater degree in the following order (1) Bell s palsy, (2) traumatic facial paralysis (3) congenital facial paralysis, (4) Melkersson Rosenthal syndrome

We shall continue the investigation of this interesting problem in future human cases and in laboratory animals, in order to establish the degenerative behaviour of the facial nerve

RÉSUME

Les auteurs decrivent les decouvertes macroscopiques observees dans "10 cas de paralysie de Bell dans un cas de paralysie faciale traumatique dans un cas de paralysie faciale congenitale et dans un syndrome de Melkersson Rosenthal On a aussi étudie avec le microscope electronique la corde du tympan de 5 malades affectés d otosclérose Nos decouvertes ultrastructurales semblent confirmer que la corde du tympan présente un comportement degenératif semblable dans tous les cas que nous avons étudies Le processus des changements dégénératifs va d une moindre à une plus grande altération et ceci dans l ordre suivant (1) paralysis de Bell (2) paralysie faciale traumatique (3) paralysie facial congenitale (4) syndrome de Melkersson Rosenthal

ZUSAMMENFASSUNG

Die Verfasser beschreiben die makroskopischen Befunde die in den 10 Fällen der Bellschen Lahmung beobachtet worden sind sowie in einem Fall der traumatischen Gesichtslahmung als auch in einem Fall von angeborener Lähmung und in einem Fall des Melkersson Rosenthal Syndroms Die Chorda Tympani dieser Patienten ist auch mit dem Elektronenmikroskop untersucht worden und man hat sie mit der Chorda Tympani von 5 Patienten die unter Oto Sklerose litten verglichen Unsere ultrastrukturellen Befunde scheinen zu bestatigen dass die Chorda Tympani ein ahnliches degeneratives Verhalten aufweist wie alle von uns untersuchten Falle Die degenerativen Veranderungen steigern sich in der folgenden Reihenfolge (1) Bellsche Lahmung (2) Traumatische Gesichtslahmung (3) Angeborene Gesichtslähmung (4) Melkersson Rosenthal Syndrom

A list of references may be obtained from the authors on request

DISCUSSION

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ACTIONS OF "LOOP" DIURETICS AND MERCURIALS UPON THE COCHLEA

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Abstract The effects of "loop" diuretics upon cochlear function are reviewed Results of recent studies on the electrophysiological, morphological and biochemical effects of perilymphatically applied diuretic and non diuretic mercurials are presented

Until recently mercurial diuretics played an important role in clinical medicine It is only in the last decade that they have been replaced by more effective compounds, in particular a chemically heterogeneous group of salidiuretic drugs, the so-called "loop" diuretics This group includes ethacrynic acid (EA), furosemide (FU), and the new compound bumetanide The name "loop" diuretics is derived from the locus of primary diuretic action, namely the thick ascending limb of the loop of Henle

If experimental animals are given "loop" diuretics, for instance EA, systemically at high but sublethal dosages, cochlear function is drastically impaired The stria vascularis (SV) seems to be the primary target of toxic action, as evidenced by a marked interstitial edema and shrinkage of intermediate cells (Quick & Duvall, 1970, Bosher et al, 1973) and by the steep (temporary) decline of the endolymphatic potential (EP) to negative values (Prazma et al, 1972) There is now general consensus that the EP is the primary electrical parameter involved, and that the changes of the cochlear microphonics (CM) constitute only a secondary effect However, considerable controversy still exists about the ionic mechanisms involved Studies by Cohn et al (1971), which indicate drastic changes of the Na–K profile of endolymph in response to low dosage of EA in the dog, have not been confirmed in other species including cat, rat, and guinea pig[1] Bosher (1976) demonstrated in the rat that changes in the Na concentration of endolymph are only of a moderate degree at a time when the EP is maximally negative due to systemic intoxication with EA (increase of Na concentration from 1 mEq/l to about 10 mEq/l) and changes in the K concentration are minimal By contrast active fluxes of these cations are severely impaired (Bosher, 1976)

In our own work in the guinea pig, we have concentrated not upon the ionic mechanisms *per se*, but rather upon changes in energy metabolism and in enzymes involved in ion transport, which should complement the ongoing work in other laboratories Most of our results on "loop" diuretics have already been published (Thalmann et al, 1973, Kusakari et al, 1976, Paloheimo & Thalmann, 1976) Therefore, we will recapitulate in this context only the most salient features, in order to provide a frame of reference for a comparison with the new results on mercurial diuretics

EA is known to interfere with a large variety of biochemical processes which can be roughly divided into alterations of energy generation,

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[1] It must be stressed however that pronounced interspecies variations in the diuretic response to EA do exist, with the dog being the most and rat the least sensitive

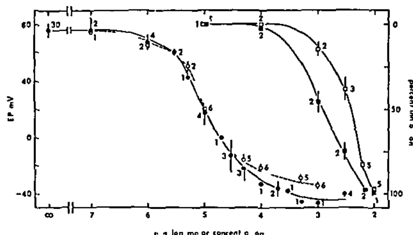


Fig 1 Effect of perilymphatically perfused ethacrynic acid upon the endolymphatic potential (● left ordinate) and upon the *in vitro* activity of strial adenylate cyclase (with 7 min preincubation ○) and NaK–ATPase (with [■] and without [□] preincubation of 30 minutes) Data points represent means ±S E M for the indicated number of ears studied If no S E M is shown its value does not exceed the size of the symbol (Adapted from Kusakari et al 1976 and Paloheimo & Thalmann 1976)

energy utilization and membrane permeability From the dynamics of strial ATP and P creatine in EA intoxication a severe alteration of energy utilization can be inferred (Thalmann et al, 1973) [1] *This finding in itself was consistent with* the originally prevailing concept that interference with NaK–ATPase is a primary mechanism responsible for the functional changes due to EA However, as evident from Fig 1, inhibition of strial NaK–ATPase *in vitro* occurs at concentrations *500 times higher than those required to* inhibit the EP by local administration of the ˬ (Kusakari et al, 1976) This is in sharp contrast to the situation with ouabain where the two inhibition curves overlap (Kuijpers, 1969) It is, therefore, extremely unlikely that NaK–ATPase is (directly) *involved in the ac*tion of EA upon the ear This is further supported by results on ^{14}C-EA, which indicate that the SV does not accumulate the drug at a time when the depression of the EP is maximal (Paloheimo & Thalmann, 1976) NaK–ATPase is not inhibited by FU at highest practicable concentrations Electrophysiological investigations by Sellick & Johnstone (1975) support these deductions

Another enzyme involved in the control of water and electrolyte transport which has been implicated as one of the targets of EA and FU action in the kidney and other transport system is adenylate cyclase (Ebel, 1974) We found th strial adenylate cyclase is strongly inhibited b EA *in vitro* and that with seven minutes of pre*incubation the inhibition curves of the EP an* of adenylate cyclase with respect to EA overla ($I_{50} = 1 \times 10^{-5}$ M, Fig 1) Inhibition by FU i pronounced even without preincubation (Paloheimo & Thalmann, 1976) These data are consistent with the proposition that interferenc *with adenylate cyclase is an important mode o* action of the "loop" diuretics However, it remains to be proved that the situation existing *in vitro* also prevails *in vivo*

Our interest in (diuretic) mercurials relates to several features they have in common with the '*loop diuretics, for instance, the susceptibility* of renal adenylate cyclase (Ebel, 1974, Jakobs et al, 1972) and NaK–ATPase (Duggan & Noll 1972, Nechay, 1973, 1974) to these agents and the fact that the primary locus of renal action seems to be the same in both groups of drugs *In view of the pronounced effects of* loop diuretics upon cochlear function it appeared relevant to determine the action of mercurial diuretics upon the inner ear and specifically upon the SV, which appears to have several features in common with kidney Mercurial diuretics are known to interfere with many other enzymes in addition to those mentioned above and at high concentrations a variety of unspecific effects upon metabolism and membrane structure characteristic of mercury intoxication

[1] Our studies also indicate a minor interference with energy generation In addition the mentioned morphological changes in the SV indicate pronounced coexistent alterations of membrane permeability

become apparent[1] It has been known for some time that mersalyl interferes at low concentrations rather specifically with anion transport Recently Burg & Green (1973*a* & *b*) and Burg et al (1973) demonstrated pronounced effects of EA, FU and mersalyl upon Cl transport or permeability in isolated perfused segments of the thick ascending limb of the loop of Henle Stupp & Rauch (1966) attempted to use mersalyl as a tool to determine anion (and specifically Cl) transport in the inner ear They observed a marked reduction of ^{42}K transport from scala vestibuli to scala media and into the SV when mersalyl was perfused through scala vestibuli for 5–10 min at concentrations ranging from 10^{-4} to 10^{-1} M The effect was more pronounced the higher the concentration The authors interpreted their data as indicating that interference with Cl transport was the actual basis of this phenomenon, and that the reduction of K transport was only secondary to that of Cl—an argument certainly open to debate Stupp & Rauch (1966) did not observe any interference of mersalyl with sulfhydryl groups, whereas v Westernhagen (1969) found a significant reduction of protein bound sulfhydryls of the cochlea in chronic intoxication with $HgCl_2$

To our knowledge no electrophysiological studies on the cochlear effects of mercurials have been reported The objective of this paper, therefore, is to describe electrophysiological changes due to perilymphatically applied diuretic and nondiuretic mercurials In addition, morphological results at the light microscopic level and biochemical studies analogous to those carried out with the "loop" diuretics will be described At this point our results are very incomplete, however, certain characteristic patterns have already emerged and we feel that a preliminary report is indicated

METHOD

Young guinea pigs weighing 200–250 g were anesthetized with pentobarbital and respired artificially after immobilization with gallamine triethiodide The bulla tympanica was exposed by the usual ventrolateral approach The mercurials used were $HgCl_2$, the nondiuretic compound *p* hydroxymercuribenzoate (PHMB), and the diuretics mersalyl and mercaptomerin These substances were dissolved in artificial perilymph and perfused through both perilymphatic scalae, as described by Kusakari et al (1976) The EP was measured by a technique similar to that described by Bosher & Warren (1968), except that the endolymphatic space was approached via the round window The organ of Corti potential (OCP) was measured by the same approach Since the OCP could only be measured for relatively brief periods of time, the data shown in Figs 3–6 represent single samplings, with two to four samplings per experiment (see Kusakari et al, 1976) The CM were recorded via an electrode in scala tympani of the basal turn A continuous pure tone of 2 300 Hz was applied in a closed system at an SPL of 60 dB re 0 0002 μbar (as measured at the tympanic membrane with the bulla open) After suitable amplification the CM were displayed simultaneously on an oscilloscope and a level recorder At intervals input/output functions were determined, using 100 msec tone pips at a duty cycle of 10%

When chemical analyses were to be performed, the cochleae were frozen rapidly, freeze dried *in toto*, and the OC and SV dissected and weighed (Thalmann, 1976) ATP and P-creatine were determined in oil wells by "enzymatic cycling" (Lowry & Passonneau, 1972) ATPases were determined by a catalytic fluorometric assay (Ahlstrom et al, 1975) Adenylate cyclase was measured by a radioimmunochemical technique (Paloheimo & Thalmann, 1976)

For morphological studies the cochleae were perfused upon conclusion of the experiments

[1] The neurotoxicity of mercury has been known since the middle ages and vertigo deafness and tinnitus due to mercury has been reported as early as 1531 by Serapius (quoted from v Westernhagen 1969) Presently organic mercurials constitute perhaps the most important environmental health hazard in Japan and several reports about the auditory and vestibular disturbances in such patients have been published Recently Falk et al (1973) demonstrated in the guinea pig that chronic administration of methyl mercury leads to a slight, but statistically significant loss of outer hair cells of the OC

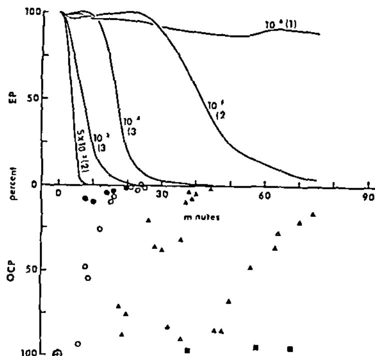


Fig 2 Effect of locally applied mercuric chloride in the indicated molar concentrations upon the endolymphatic potential (EP) and the organ of Corti potential (OCP) Absolute values in mV have been converted to percentages with 'zero time' values equal to 100 percent ($EP_0 = 87\,1 \pm 5\,9$ mV, $OCP_0 = -82\,9 \pm 6\,0$ mV, $n = 11$ in each case) EP tracings represent the mean of number of experiments in parentheses The OCP measurements represent individual samplings with two to four samplings per experiment OCP symbols ● = 5×10^{-3} M, ○ = 10^{-3} M ▲ = 10^{-4} M △ = 10^{-5} M ■ = 10^{-6} M

with a buffered solution of 1 % osmium tetroxide, followed by dehydration and embedding *in toto* in Araldite (Bohne, 1972)

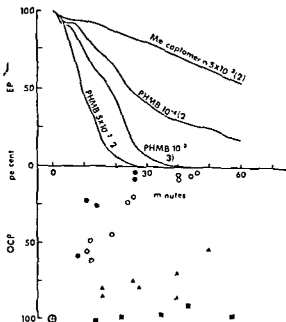


Fig 3 Effect of locally applied *p*-hydroxymercuribenzoate (PHMB) and mercaptomerin upon the EP and OCP EP_0 and OCP_0 for PHMB are $82\,1 + 6\,5$ mV ($n = 7$) and $-86\,0 \pm 5\,0$ mV (n 7) respectively For details see Fig 2 OCP symbols ● = PHMB 5×10^{-3} M ○ = PHMB 10^{-3} M ▲ = PHMB 10^{-4} M ■ = mercaptomerin 5×10^{-3} M

RESULTS AND DISCUSSION

Figs 2 and 3 demonstrate the changes of th EP and the OCP due to $HgCl_2$ and the organi nondiuretic PHMB, respectively At higher con centrations both compounds rapidly eliminat the EP, however, in contrast to the 'loop diuretics and ouabain the EP does not turn nega tive Whereas the OCP remains normal in sys temic EA intoxication (Thalmann et al, 1975; Honrubia et al, 1976), in local intoxication with mercurials it is abolished at a rate similar to that of the EP[1] Qualitatively similar results are seen with the diuretic mersalyl, except that on a molar basis higher concentrations are required to effect electrophysiological changes compar able to those produced by $HgCl_2$ (Fig 4) In addition, a highly reproducible initial increase of the EP is visible and the OCP does not drop all the way The other diuretic mercurial, mer captomerin, produces only moderate electro-physiological changes (Fig 3) Fig 5 shows pre-liminary CM recordings for $HgCl_2$ and mersalyl applied at 1×10^{-3} M It is seen that the CM

[1] The OCP has not yet been determined when EA FU or ouabain were applied locally However the EP ex hibits a pronounced negativity in all three instances

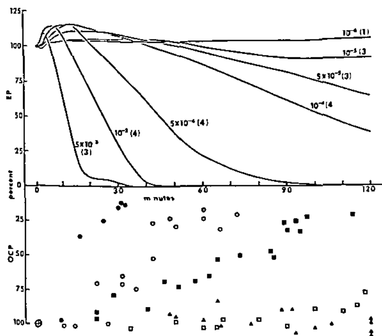


Fig 4 Effect of locally applied mersalyl upon the EP and OCP EP_0 and OCP_0 are 87 7±4 4 mV (n=22) and −83 9±5 6 mV (n=21), respectively For details, see Fig 2. OCP symbols ● = 5×10^{-3} M, ○ = 10^{-3} M, ■ = 5×10^{-4} M, □ = 10^{-4} M, ▲ = 5×10^{-5} M, △ = 10^{-5} M

drops at an even faster rate than either the EP or the OCP.

Kuijpers (1969) proposed that the normal EP is the resultant of two independent potentials of opposite polarity, (1) A large positive potential of 100 to 120 mV, thought to be due to electrogenic K transport into scala media (Sellick & Bock, 1974), and (2) a smaller negative component (−20 to −40 mV), thought to be a diffusion potential across Reissner's membrane The positive potential is extremely susceptible to metabolic interference and is rapidly abolished in anoxia and intoxication with ouabain and "loop" diuretics, whereas the negative K diffusion potential declines only very gradually. Therefore, because of the fundamentally different time courses, the negative diffusion potential becomes manifest Whereas there seems

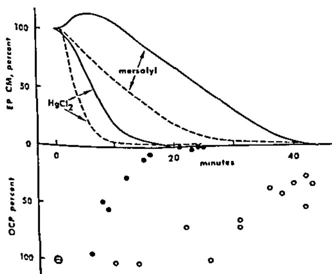


Fig 5 Effects of locally applied mersalyl (○) and $HgCl_2$ (●), both at 10^{-3} M concentration, upon EP (——), cochlear microphonics (CM) (- - -), and OCP All are expressed in percentage of original, "zero time", level EP_0 (mersalyl) = 88 6±4 6 mV (n=4) OCP_0 (mersalyl) = −80 5±4 8 mV (n 4) EP_0 ($HgCl_2$) = 89 5±4 9 mV (n=2), OCP_0 ($HgCl_2$) = −83 5±9.2 mV (n 2)

to be general consensus about the nature of the positive electrogenic potential, there exists considerable controversy about the postulated negative K diffusion potential Honrubia et al (1976) presented arguments against the existence of this potential and proposed that the negative EP is due to persistence of the OCP The OCP declines only slowly in anoxia and not at all in acute systemic intoxication with "loop" diuretics The electrophysiological changes due to mercurials (Figs 2-5) could be interpreted as supporting the viewpoint of Honrubia et al (1976) It could be argued that, (1) since in contrast to most other types of interference, the OCP drops as rapidly as the positive electrogenic potential, the EP has no chance to turn negative, and that, (2) the even more rapid elimination of the CM is due to the breakdown of both cochlear "batteries" However, the failure of the EP to turn negative could equally well be explained by a concomitant decline of the postulated K diffusion potential across Reissner's membrane [1] In fact, considering the well known deleterious effects of the mercury ion upon biological membranes, it was d likely that the mercurials lead to massive unspecific alterations of permeability of inner ear membranes It was even conceivable that the (positive electrogenic component of the) EP may collapse in the absence of alterations of its generator in the SV *per se* Before engaging in complex electrophysiological studies on alterations of electrical impedances, or in determinations of ion distributions and fluxes, we initiated morphological studies to determine whether or not any gross membrane alterations were present

The cells in the inner ear found to be most sensitive to mersalyl were the outer hair cells (OHC) In cochleae perfused for 30 min with 1×10^{-4} M mersalyl the OHC bodies were moderately swollen, but their plasma membranes were still intact Nonmyelinated nerve fibers in the inner spiral bundle had also begun to swell The rest of the cells comprising the OC, SV, and Reissner's membrane had normal appearance as judged by light microscopy

With perfusion of 5×10^{-4} M mersalyl for 30 min, a moderate number of OHC were in the process of degenerating (Fig 6) These cells were characterized by enlarged, pale staining nuclei, vacuolated cytoplasm and ruptured plasma membranes The epithelial cells of Reissner's membrane (RM) appeared to have a slightly increased number of vacuoles in their cytoplasm The junctions between the cells were normal, however

In specimens perfused for 30 min with 1×10^{-3} M mersalyl, most OHC were in the process of degenerating [2] However, the apical membrane of all of these cells was still present The remaining cells of the OC were reasonably well preserved In the SV, the intercellular spaces between marginal and intermediate cells were considerably widened, while the cytoplasm of the intermediate cells had shrunk (Fig 7*C*) In cochleae perfused for 30 min with 5×10^{-3} M mersalyl, the reticular lamina of the OC was still intact However, all OHC had degenerated and the supporting cells were so swollen that all fluid spaces in the OC were obliterated The epithelial cells of Reissner's membrane (RM) did not appear significantly different from those in the 5×10^{-4} M mersalyl specimens In the SV, in addition to the changes described for perfusion with 1×10^{-3} M mersalyl, there was vacuolization of some of the marginal cells and

[1] In this context the initial increase of the EP in the early stage of mersalyl intoxication should be discussed (Fig 4) An increase of the EP has been seen when either the postulated negative K diffusion potential or the OCP were abolished (Kuijpers 1969 Honrubia et al 1976) In our experiments the OCP does not appear to be decreased at times when the EP is maximally increased by mersalyl (Fig 4) It is conceivable that the increase of the EP is due to the specific inhibitory effect of incipient mersalyl intoxication upon Cl (and K) permeability of Reissner's membrane as postulated by Stupp & Rauch (1966)

[2] It should be noted that in our measurements of the OCP, the electrodes (tip diameter 2 3 μm) penetrate in the region of the supporting cells lateral to the hair cells However, with more refined techniques it is now possible to measure the potential of the outer hair cells

tional way



Fig 6 Cochlea perfused for 30 min with 5 10^{-4} M mersalyl (A) Phase contrast photomicrograph showing normal reticular lamina and stereocilia on outer hair cells and inner hair cells (B) Focused below reticular lamina in (A) Some of the swollen outer hair cells have degenerating nuclei (*D*) and their plasma membrane are only faintly visible (C) Stained 1 μm section through [illegible] (A) and (B) [illegible] remains Rest of cells of organ of Corti still have normal appearance


A
M
V


B


C


D
5μ

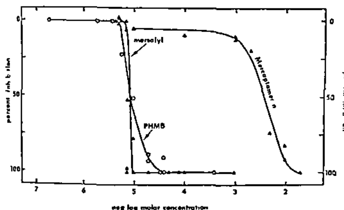


Fig 8 Inhibition of strial NaK–ATPase by the mercurials mersalyl (▲), *p*-hydroxymercuribenzoate (PHMB, ○), and mercaptomerin (△) Each data point represents the mean value of four determinations from a single experimental ear

partial detachment of the SV from the spiral ligament (Fig 7*D*) The changes in the SV appear similar to those found by Quick & Duvall (1970) in EA intoxication, but a detailed comparison will be possible only by electron microscopy

With regard to the biochemical mechanisms involved, we approached the problem in a way similar to that used in the study of the "loop" diuretics Because of the marked effects of mercurials upon renal NaK–ATPase (Nechay, 1973) we studied this enzyme first Fig 8 demonstrates that mersalyl inhibits strial NaK–ATPase at a remarkably low concentration ($I_{50}=8\times10^{-6}$ M) which is only moderately higher than that required with ouabain ($I_{50}=3\times10^{-6}$ M) Note the extreme steepness of the inhibition curve The pronounced inhibition of NaK–ATPase is in sharp contrast to the earlier described situation with "loop" diuretics The inhibitory effect of the nondiuretic PHMB is equally marked, but it is known that the inhibition of renal NaK–ATPase *in vitro* is independent of whether a particular mercurial is diuretic or nondiuretic (Nechay, 1973) The effect of mercurials upon strial NaK–ATPase is highly specific, Mg-ATPase is only slightly inhibited at highest concentrations This again is in contrast to EA, in which case the inhibition of NaK–ATPase is accompanied by a considerable inhibition of Mg–ATPase (Kusakari et al, 1976) Significant inhibition of NaK–ATPase by mercaptomerin occurs only at high concentrations, in line with the moderate effects of this drug upon the EP (Fig 3)

Fig 7 One micron sections of stria vascularis (*SV*) from middle of first turn (A) Control ear perfused for one hour with artificial perilymph Marginal (*M*) intermediate (*I*) and basal cells (*B*) visible V intra-epithelial capillary (B) Perfused for 30 min with $5\cdot10^{-4}$ M mersalyl Beginning enlargement of intercellular spaces (*arrows*) (C) Perfused for 30 min with $1\cdot10^{-3}$ M mersalyl. Intermediate cells (*I*) quite shrunken and intercellular spaces (*arrows*) have widened considerably (D) Perfused for 30 min with $5\cdot10^{-3}$ M mersalyl In addition to findings described in (C) intracellular vacuoles (*small arrows*) are present in some marginal cells Also SV had begun to detach (*at large arrow*) from spiral ligament.

Pronounced effects of mersalyl upon adenylate cyclase have been observed in cortex and medulla of the rat kidney (Jakobs et al, 1972) We found that adenylate cyclase of the SV and the OC is inhibited by mersalyl at even lower concentrations than NaK ATPase ($I_{50}-5\times10^{-6}$ M and 9×10^{-7} M, respectively, Thalmann et al, 1976)

In principle, the high susceptibility either of strial NaK ATPase or of adenylate cyclase to mersalyl could alone account for the observed alterations of the EP It must be noted, however, that effects upon the EP occur only at much higher concentrations This discrepancy between *in vitro* and *in vivo* results is as yet unexplained

At this point only preliminary results are available concerning the changes of high energy phosphates due to mercurials in SV (Table I) and OC (not shown) Following perfusion of 1×10^{-3} M $HgCl_2$ for 20 min, i e, at a time when all cochlear potentials are abolished, both ATP

Table I *Steady state levels of ATP and P-creatine in the stria vascularis of guinea pigs following perilymphatic perfusion of mercurials at the indicated concentrations and durations*

The contralateral ears were used as controls The units are moles per kg dry tissue Data represent the means ±S E M for 8–10 samples of stria vascularis from the first and second cochlear turn

	ATP		P-creatine	
	Control	Experimental	Control	Experimental
$HgCl_2$ 1×10^{-3} M 20 min	14 8±1 0	3 8±0 9[a]	10 1±0 7	2 3±0 3[a]
$HgCl_2$ 1×10^{-3} M 10 min	14 6±0 7	9 4±1.3[a]	8 9±0 8	4 0±0 6[a]
Mersalyl 5×10^{-4} M 30 min	16 2±0 9	17 6±0 7[b]	11 6±0 7	10 9±0 9
Mersalyl 5×10^{-4} M 30 min	13 0±0 7	12 5+0 6[c]	7 5±0 5	6 4±0 3[a]
Mersalyl 1×10^{-3} M 30 min	14 1±0 9	13 4±0 6	10 4+0 6	3 6±0 8[a]
Mersalyl 1×10^{-3} M [illegible] min	13 5±0 6	13 7±0 7	7 8±0 9	3 8±0 3[a]
[illegible]yl 1×10^{-3} M 50 min	14 7±1 2	5 2±0 5[a]	7 6±0 8	4 0±0 6[a]

[a] $p<0.001$ [b] $p<0.01$ [c] $p<0.05$

and P creatine are strongly reduced in SV and OC At the 10 minute mark, at which time the CM are abolished, but the EP and the OCP have not declined all the way, reduction of P creatine and ATP is still very marked This, of course, indicates a severe interference with energy generation

In the case of mersalyl, only a minimal reduction of high energy phosphates is seen in the OC following perfusion at 5×10^{-4} M for 30 min No (biologically significant) changes are present in the SV, however electrophysiological alterations are only moderate at this stage (Fig 4) Following perfusion of mersalyl at 1×10^{-3} M for 30 min both high energy phosphates are significantly reduced in the OC However, since the outer hair cells are disrupted at this stage, the biochemical results on the OC are of limited value In the SV, ATP levels are still normal at this stage but P-creatine is reduced by about 60% A selective reduction of P-creatine in the presence of normal ATP levels is a characteristic early sign of interference with energy generation At 50 min of perfusion, at which time electrophysiological changes are maximal, ATP levels as well are markedly reduced in the SV

Whereas the behavior of high energy phosphates in pronounced mercurial intoxication is indicative of an interference with energy generation, a coexistent interference with energy utilization cannot be singled out without evaluation of the dynamics of high energy phosphates in superimposed ischemia (Thalmann et al, 1973) No such studies have been carried out to date, however, the pronounced susceptibility of strial NaK–ATPase (and adenylate cyclase) to mercurials would suggest that energy utilization is also interfered with

From the presented biochemical data it is apparent that the mechanisms of action of locally applied mercurials upon the inner ear are very complex In addition, the morphological findings (Figs 6 & 7) indicate gross changes of some cochlear membranes, which would in themselves account for the abolition of the cochlear potentials However, from these findings alone it was not clear to what extent the strial generator of the EP *per se* is involved and to what extent the decline of the EP could be explained by the breakdown of membranes of other structures bounding the cochlear duct This could be tested by application of mercurials via the vascular route, since it is reasonable to assume that their concentration would be at least initially much higher in the SV than in the cochlear fluids In contrast to the 'loop diuretics, systemic administration of mercurial diuretics at high concentration is precluded by pronounced effects upon the heart and other vital organs However, when perfusing 5×10^{-3} M mersalyl via the anterior inferior cerebellar artery in the surviving inner ear (Wada et al,

1976) the EP does turn negative (as much as −18 mV) which suggests that the generator of the EP is inhibited at a time when alterations of other structures bounding scala media probably are minimal

RÉSUME

Les effets des diuretiques « loop » sur la fonction de l'oreille interne sont examines. Des resultats des nouvelles recherches electrophysiologiques, morphologiques et biochemiques sur l effet des substances mercurielles diureti ques et nondiuretiques appliquees par perfusion peri lymphat que sont presentes

ZUSAMMENFASSUNG

Der Einfluß von Loop Diuretika auf die Funktion der Cochlea wird besprochen Ergebnisse neuerer Unter suchungen uber elektrophysiologische morphologische und biochemische Effekte von perilymphatisch verabreichten diuretischen und nichtdiuretischen Quecksilber verb ndungen werden berichtet.

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ELECTRON PROBE DETERMINATION OF RELATIVE ION DISTRIBUTION IN THE INNER EAR

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Abstract The relative amounts of potassium and chlorine present in various parts of the inner ear were studied by using an energy dispersive X ray spectrometer in conjunction with a scanning electron microscope. Whereas the amount of chlorine was high in all compartments investigated potassium was high in endolymphatic spaces and low in perilymphatic spaces. High contents of potassium and chlorine were also found in the tectorial membrane in the inner sulcus and in the cupula of the semicircular canal. It is concluded that the tectorial membrane and the cupula do not present a barrier to ions and that therefore the sensory hairs in the inner ear are exposed to the ionic environment provided by the endolymph

The endolympahtic fluid of the inner ear is unique as an extracellular fluid in having a particularly high potassium content. The ionic composition of inner ear fluids has been determined by chemical analysis of samples of endolymph and perilymph collected by micropipette (Smith et al., 1954, Citron et al 1956, Silverstein, 1966, Bosher & Warren, 1968). The ionic content has also been recorded in the cochlear partition by ion-selective electrodes (Suga et al 1970 Sellick & Johnstone 1975). These measurements agree that endolymph is high in potassium about 150 mEq/l, and low in sodium content about 6 mEq/l (Fig 1). Chlorine is high about 115 mEq/l. Such ionic environment is not compatible with the functioning of neurons and is surprising in view of the fact that the receptor cells of the inner ear sensory epithelia have their apical membranes facing the endolymph. In the cochlea, however, the sensory hairs do not project directly into the endolymph but are separated from it by the tectorial membrane which provides the necessary mechanical stimulus. It has been argued that the tectorial membrane constitutes a barrier to ions and that the sensory hairs are bathed in a fluid

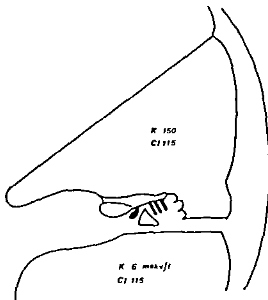


Fig 1 Schematic drawing which illustrates the approximate proportions of potassium and chlorine present in the endolymphatic (*hatched*) and perilymphatic space of the cochlea.

This work has supported by grant no 04x-02461 from the Swedish Medical Research Council and was carried out at the King Gustaf V research institute Stockholm





Figs 2 and 3 The three scalae of the cochlea appear empty after conventional fixation procedures (*left*) but are found to contain abundant material after freeze-drying without prior fixation (*right*)

more similar to a normal extracellular environment (Naftalin, 1965, Saito, 1967, Lawrence, 1967) This viewpoint is maintained in par by Lawrence et al (1967, 1974), who that a microelectrode which is passed the organ of Corti measures zero al inside the tectorial membrane as opposed to the +70 mV of the scala media

The present work is directed at this question and uses a scanning electron microscope equipped with an energy dispersive X ray spectrometer to map the relative distribution of potassium and chlorine in discrete areas within the inner ear

The image produced in the scanning electron microscope is formed by a narrow beam of electrons which sweeps the specimen The high energy primary electrons of the beam knock out electrons from atoms in superficial layers of the specimen These so-called secondary electrons are used to form the image The method of analysis used here exploits another phenomenon which also occurs when the primary electrons collide with atoms the absorbed energy is emitted as electromagnetic radiation in the X ray region Since the wavelength is proportional to the atom number of each excited element, an X ray spectrometer can be used in conjunction with the scanning microscope to analyse the presence of various elements in areas which are simultaneously viewed on the microscope screen

This method has been used in the present investigation to determine the relative distribution of potassium and chlorine in the tectorial membrane, in the inner sulcus and in the cupula of the semicircular canal in reference to that of the endolymphatic and perilymphatic spaces The results have been summarized in a preliminary report (Flock, 1973)

MATERIALS AND METHODS

Five guinea pigs were anesthetized with chloroform the temporal bones were taken out rapidly, and the bulla was opened Without further dissection and with no fixation the temporal bones were quenched in liquid propane cooled to −190°C by liquid nitrogen The frozen specimens were transferred to

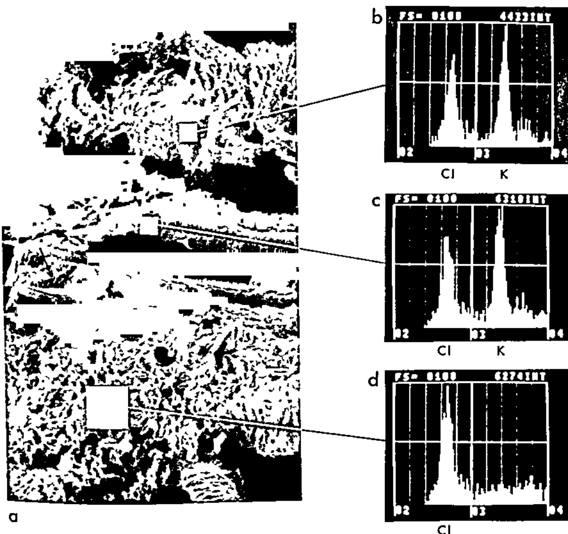


Fig 4 (*a*) A segment of cochlear partition is seen in the scanning electron microscope The modiolus has been removed and the material of the scala media (*above*) the organ of Corti with the tectorial membrane (*centre*) and the scala tympani (*below*) are viewed from the modiolar side The histogrammes of ion content presented in (*b*) (*c*) and (*d*) are obtained by analysis of the regions indicated by the corresponding white squares

a metal plate submerged under liquid nitrogen where the temporal bones were fractured along desired cleavage planes with a chisel In this way internal structures of the inner ear were exposed The specimen were then freeze-dried at −30°C, glued to specimen stubs for scanning electron microscopy and covered with a thin layer of gold by vacuum evaporation

The specimen were examined in a Cambridge Stereoscan scanning electron microscope and suitable areas were chosen for analysis of ion content This was done by energy dispersive X-ray spectroscopy (Edax) The combined instrument is referred to as an electron probe

RESULTS

Freeze drying of non-fixed tissue

Conventional techniques for preparing the inner ear for structural analysis usually involve fixation of the cochlea and vestibular



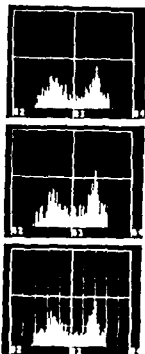


Fig 5 The three histogrammes of ion distribution at the right have been sampled from the regions marked by white squares in the scanning picture to the left from above to below endolymphatic material of scala media membrana tectoria inner sulcus

labyrinth by local perfusion The fluid spaces of the inner ear then seem to be devoid of structural material A freeze fracture through a cochlea treated in this way is seen in Fig 2 The perilymphatic spaces of scala vestibuli and scala tympani, as well as the endolymphatic space of the scala media appear empty The freeze dried non fixed cochlea seen in Fig 3 has quite a different appearance, all the three scalae are now seen to be filled with desiccated material The nature of this material is unknown but is assumed to consist of proteins, glycoprotein or mucopolysaccharides (Giebel & Wespi, 1973) which are dissolved in the endo and perilymph *in vivo* but have precipitated during the freeze drying process The tectorial membrane and organ of Corti are preserved in considerable detail (Figs 4 and 5)

Electron probe analysis

Analysis was done in descrete areas by magnifying at desired points While the beam scanned such areas, three of which are marked by white squares in Fig 4, the X-ray spectrometer counted the number of emitted quanta and sorted them according to their different energies The histogrammes in Figs 4 and 5 are obtained in this manner

Analysis of *endolymphatic material* (Fig 4*b*) yields two peaks, one centered around 2 65 keV and the other around 3 38 keV These peaks are identified as the k_α emission of chlorine and potassium respectively

Analysis of *perilymphatic material* (Fig 4*d*) gives only one peak, that for chlorine

Analysis of the tectorial membrane shows peaks for potassium and chlorine in the same relative proportions as in endolymphatic material (Fig 4*c*)

Fig 5 presents an analysis of material contained in the *inner sulcus* as well as of the tectorial membrane and endolymphatic material All these regions contain the same relative amounts of potassium and chlorine

Examination of the *ampulla of the semi*

circular canal gave similar results in the sense that the substance of the cupula contained potassium and chlorine in proportions similar to endolymphatic material whereas perilymphatic material lacked potassium

DISCUSSION

With the present method, the content of potassium and chlorine is found to be high in the endolymphatic space whereas chlorine is high and potassium is low in the perilymphatic space This is in agreement with the ionic distribution established with other methods (see Bosher & Warren, 1968) It appears that during the freeze-drying procedure the ions present in the fluid compartments become trapped in the meshwork of material which precipitates so that the ionic distributions in this material reflect the situation *in vivo*

From a physiological point of view the main issue is whether or not the sensory hairs are exposed to the potassium-rich environment of the endolymph The sensory hairs extend from the lamina reticularis, where the apical ends of the receptor cells are locked to the lower surface of the tectorial membrane where the tips of the sensory hairs are attached (Kimura, 1966, Lim, 1972, Engstrom & Ades 1973) This space is called the subtectorial space and communicates with that of the inner sulcus The present results indicate that this space does in fact have the same high potassium content as the endolymph and that the ions of the endolymph can get access to the subtectorial space by diffusion through the tectorial membrane This does not exclude the existence of an additional pathway through a slit between the outer lip of the tectorial membrane and the Hensen cells (Helle 1974)

This finding is in conflict with that of Lawrence et al (1974) and to some extent with Ross (1975) who both suggest that the tectorial membrane is impermeable to ions and forms a barrier which separates the subtectorial space from the endolymph Lawrence et al (1974) found that a microelectrode which passes from scala tympani to scala media through the organ of Corti, traverses a space of zero potential before it encounters the +70 mV endocochlear potential of the endolymphatic space The border is suggested to be located at the upper surface of the tectorial membrane Ross (1975) concludes from her work also with an electron probe, that there is a gradient of ions within the tectorial membrane potassium being diminished towards the subtectorial space The areas of analysis and incident angle of the beam are not documented

The claim of Lawrence et al (1974) for zero potential inside the tectorial membrane has been disputed by other authors (see Fex, 1974, pp 602) Also the assertion that the tectorial membrane must be impermeable to ions is in conflict with a number of other investigations in which the communication between fluid compartments in the cochlea have been studied in the electron microscope by the use of electron dense molecular tracers Ilberg (1968) and Angelborg (1976) used thorotrast and Rudert (1969) and Jahnke & Rudert (1973) used ferritin, which has a molecular weight of 750 000, as tracers In all cases tracer molecules were found inside the tectorial membrane Tonndorf et al (1976) found that vital dyes readily penetrate the tectorial membrane

In the basilar papilla of lizards certain regions of sensory epithelium are not covered by tectorial membrane, here the sensory hairs are exposed directly to endolymph (Miller, 1974 Bagger Sjoback & Wersäll 1973) This is true also in the cochlea of birds where the sensory hair bundles project into wide canals in the tectorial membrane which open to the endolymphatic space (Tanaka & Smith, 1975) In these cases it seems that hair cells can function with their apical ends in a high potassium environment High potassium has recently been registered with ion selective electrodes within the cupula of free standing lateral line organs (Russell & Sellick, 1976), and in insect mechanoreceptors Thurm (1974) makes a case for high potassium content as a crucial factor in mechanosensitivity

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ZUSAMMENFASSUNG

Mittels eines energiedispersiven Rontgenspektrometers und in Verbindung mit einem Rasterelektronenmikroskop wurde die relative Menge von Kalium und Chlor in verschiedenen Teilen des Innenohres untersucht Wahrend der Chloridgehalt in allen untersuchten Be reichen hoch war war der Gehalt an Kalium hoch in den endolymphatischen und niedrig in den perilympha tischen Raumen Eine große Menge Kalium und Chlor wurde auch in der Membrana tectoria im inneren Sulcus und in der Cupula des Bogenganges beobachtet Die Be funde weisen darauf hin daß die Membrana tectoria und die Cupula für die Ionen kein Hindernis darstellen und daß daher das Ionenmilieu um die Sinneshaare des Innenohres der Endolymphe entspricht

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VISUAL SUPPRESSION OF CALORIC NYSTAGMUS IN NORMAL INDIVIDUALS

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Abstract The effects of opening of the eyes and of ocular fixation upon caloric nystagmus were investigated during the period of maximum intensity of caloric nystagmus in a series of 32 normal individuals The percentage reduction in slow phase velocity induced depended upon the test conditions but on the other hand did not depend upon the temperature of the water applied as caloric stimulus This latter fact favors the theory of visual suppression of the caloric test Another striking finding was that a clear correlation definitely existed between the percentage reduction of suppression in slow phase velocity and that in the multiplication product of amplitude by nystagmus frequency (P A F) during the period of eye opening and ocular fixation The percentage of suppression in slow phase velocity is interchangeable with that in P A F which broadens the practical scope of the routine test

It is well known that caloric induced nystagmus is definitely suppressed by ocular fixation (Mahoney et al 1957, Anderson et al 1958, Sokolovski, 1966 Hart 1967, Hood & Dix, 1973, Alpert 1974, Molnar & Torok, 1974) In some instances of disorders of the central nervous system on the contrary a lesser degree of (or no) suppressive effect of ocular fixation upon the caloric nystagmus has also been recognized which state has been termed ' Failure of Fixation–Suppression of Caloric Nystagmus (FFS) (Coats, 1970), paradoxical caloric response (PCR) (Maccario et al , 1972), and also ocular fixation reversal phenomenon (Torok, 1973) Quantitative evaluation of fixation suppression of caloric nystagmus on the other hand, has also been carried out by some investigators Demanez & Ledoux developed an ocular fixation index (OFI) (1970) Alpert (1974) also indicated that OFI based on slow-phase velocity was found to be the best method of separating a normal from an abnormal fixation suppression of caloric nystagmus Maccario et al (1972) calculated an 'EO/EC ratio of caloric nystagmus, defined as the average deflection with the eyes open divided by the average deflection with the eyes closed Molnar & Torok (1974) studied the effect of fixation and non fixation upon the caloric nystagmus in both weak and strong caloric stimulation and concluded that slow phase velocity and amplitude measurements following the strong stimulus were more informative As outlined above, attention has hitherto been directed toward reliable parameters with which to test the fixation suppression of caloric nystagmus As regards test conditions, on the other hand, little attention has been paid

In the present study we have attempted to assess the standardization of our modified caloric test Particular attention was focused upon the effects of eye opening and ocular fixation upon caloric nystagmus from the following points of view (1) whether or not the effects of eye opening and ocular fixation upon

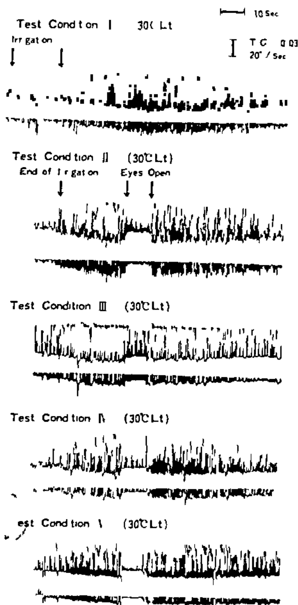


Fig 1 ENGs representing visual suppression of caloric nystagmus under each test condition in the same indi

ments to the right Upper traces in each test condition show eye velocity and bottom traces in each test condition exhibit slow phase eye velocity Calibration and time scale are indicated by the vertical and horizontal bar respectively on the upper right of most of the traces Reduction in slow phase velocity induced by eye opening and ocular fixation was dependent upon each test condition Mean percentage reduction in slow phase velocity in each test condition 69 0% 58 5% 64 1% and 84 2% respectively

caloric nystagmus are dependent upon the temperature of the water applied as caloric stimulus, (2) whether or not the degree of visual suppression of caloric nystagmus varies according to the test conditions, (3) whether or not a reduction in slow phase velocity induced by eye opening and ocular fixation can be replaced by a reduction in P A F during the period of eye opening and ocular fixation

METHOD

All subjects used were medical and para medical students, 17 females and 15 males with ages ranging from 18 to 25 years and in good general physical condition None of them had any past history of otoneurological dis orders or had any experience of exposure to caloric tests None had taken drugs or alcohol for at least 2 days prior to tests

The subjects were examined prior to tests for evidence of spontaneous nystagmus and abnormalities of tympanic membrane and audiogram

Test Procedure

In attempting to assess the influence of eye opening and ocular fixation the following test conditions were employed

Test Condition I

Control test Ad modum Fitzgerald & Hallpike (1942) modified by Otani (1962), caloric stimu lus was 50 ml water most often 30°C or 44°C, sometimes of 17°C, for an irrigation period of 20 sec with 5– min rest intervals between the end of one trial and the beginning of the next All subjects were investigated with one test condition only per day Prior to each trial, calibration was performed to adjust a pen deflection of about 20 mm per 20 degrees of eye movement A test trial was made in all subjects, with room illuminated and eyes closed All subjects were always required to perform mental arithmetic throughout the period of the trial

In 32 subjects, maximum slow phase eye

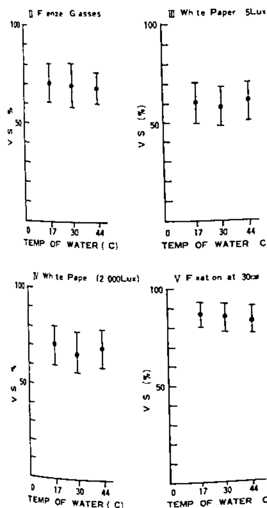


Fig 2 Mean percentage reduction in slow phase velocity induced by each test condition was established as compared with the control period (see text) The amounts of percentage reduction tended to be almost equal in each test condition without any correlation to water temperature applied as caloric test Of the four test conditions on the other hand amounts of percentage reduction were dependent upon test conditions The first was Test Condition V in which caloric nystagmus was most strongly suppressed The second was Test Conditions II and IV The third Test Condition III This classification proved significant ($p<0.01$)

velocity occurred at about 49 sec after the onset of irrigation and was maintained for about 30 sec whether the water temperature of caloric stimulus applied was 30°C or 44°C (Fig 1) The visual suppression test was carried out during this ... caloric nystagmus ... condition

Test Condition II

Frenzel glasses Mean ... slow phase eye velocity ... during the control period ... the onset of irrigation ... onset subjects were asked ... under Frenzel glasses for 10 ... close them until there was ... tagmus A comparable ... phase velocity was established ... half of the 10 sec period ... onset of irrigation) In the ... under Frenzel glasses ...

Visual suppression ... centage reduction in ... (SP Vel) (Takemori & ...

% Visual suppression ...

$$= \frac{\text{SP Vel control} \ldots}{\text{SP V} \ldots}$$

Test Condition III

Homogeneous ... which reflected ... to open their eyes ... of irrigation ... covered with the ... distance A ... phase velocity ... n same time ... ne II (Figs 1 and 2)

Test Condition ...

White paper ... by 2 000 lux ... slow that of Test ... 1966) test the surf... phase eyes was ... another evenly with ... is worth during the ... not slow tion in the ... is a para calculated ... of visual of Test Co... ve estab-

Table I *The effect of eyes opening and ocular fixation upon caloric nystagmus are investigated in a series of 32 normal individuals*

Values given are numbers of ears tested in each test condition

Test condition	Caloric stimulation		
	30°C	44°C	17°C
I Control	64	31	19
II Frenzel glasses	26	19	15
III White paper (5 lux)	31	22	17
IV White paper (2 000 lux)	28	21	17
V Fixation at 30 cm	29	24	17

Test Condition V

Fixation Subjects were asked to open their eyes and fix the gaze upon the examiner s index finger tip about 30 cm away during the same time course as that of Test Condition II

A comparable value in the slow phase velocity induced by ocular fixation was eval uated as described in Test Condition II (Figs 1 and 2)

Cold stimuli from 17°C to 20°C were used to induce stronger caloric nystagmus in 19 subjects picked up randomly in order to deter- whether or not reduction in slow phase y induced by each test condition is dent upon the intensity of caloric stimuli (Table I) In 19 subjects, maximum slow phase velocity occurred at about 45 sec after the onset of irrigation and lasted for about 30–40 sec The effect of eyes opening and ocular fixation upon the caloric nystagmus was tested during the same time course as that of Test Condition II according to each test condition (Table I, Fig 2) Comparable values and percentage reduction in the slow phase veloc ity induced were established in each test condition, as described in Test Condition II (Fig 2)

Registration

For all trials the subjects heads were elevated 30° from the horizontal position San-ei electronystagmography apparatus was used to record horizontal eye movements from the electrodes taped near the outer canthus of each eye A ground electrode was located on the forehead

Standard deviation, and correlation between each test condition were statistically evaluated using PDP-12 computer

RESULTS AND DISCUSSION

Fig 2 represented mean percent reduction in slow phase velocity and its standard deviation of caloric nystagmus induced by each test condition One of the striking features was that the percentage reduction in each test condition was not dependent upon the water temperature applied as caloric stimulus on one side, but, on the other side, dependent upon test condition Under Test Condition V, for instance, the mean percentage reduction was 84 2% for 30°C, 81 7% for 44°C, and 86 8% for 17°C of caloric stimulus respectively of which values tended to be almost equal with out any correlation of water temperature used as caloric stimulus ($p<0\ 01$) (Fig 2) Among four test conditions represented in Fig 2 how ever, the mean percentage reduction for 30°C of caloric stimulus, for instance varied ac cording to each test condition i e, 69% for Test Condition II, 58 5% for Test Condition III, 64 1% for Test Condition IV, and 84 2% for Test Condition V respectively According to the intensity of percentage reduction these test conditions were classified into the following three categories The first was Test Condition V, in which caloric nystagmus was most strongly suppressed The second was Test Conditions II and IV, between which no significance was detected in the amounts of percentage reduction ($p>0\ 05$) The third Test Condition III, proved significant (Fig 2)

In attempting to assess the influence of light levels upon caloric nystagmus we employed three phototopic light levels Between Test Conditions II and IV, however, no statistic significance could be found ($p>0\ 05$) The dif ference in the percentage reduction between Test Conditions III and IV, on the other hand, was significant ($p<0\ 01$) (Fig 2) This dif

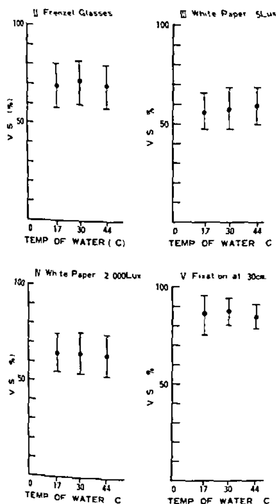


Fig 3 The amounts of suppression in P A F induced by each test condition The percentage reduction in P A F also tended to be nearly equal in each test condition without any correlation to water temperature used as caloric stimulus though the intensity of suppression in P A F was dependent upon each test condition The percentage reduction in P A F in each test condition on the other hand closely paralleled the percent reduction in slow phase velocity in each test condition (see Fig 2)

ference is considered as ascribable to the light levels, as the test procedures for both test conditions were the same, except for light levels

Under Test Condition III, in which subjects were not required to fix their gaze the mean percentage reduction was 58 5% for 30°C, 60 2% for 44°C, and 59 9% for 17°C of caloric stimuli respectively, i e about 20% lower than that under Test Condition V The amounts of percentage reduction even under Test Condition III were very close to 50% This approximated the amounts of visual suppression of caloric nystagmus studied in rhesus monkey (Takemori & Cohen, 1974) Thus, there exists a definite difference in percentage reduction in slow phase velocity between human beings and monkeys This difference might be largely due to the fixation function of the species This speculation could be supported by the fact that in vertebrates such as rabbits and cats, which can be presumed to have poor fixation ability, visual suppression of caloric nystagmus was very slight (unpublished, Kato & Aoyagi, 1975)

In the present study, the difference in amounts of percentage reduction proved significant between Test Conditions III and V ($p<0\ 01$) (Fig 2) This difference in intensity of visual suppression might be due to whether there is active participation in fixation function (or lack of it), as under Test Condition III, the subjects' eyes were covered with white paper about 10 cm apart and subjects were not put in such a situation as to exert their fixation function Under Test Condition V, on the other hand, subjects were asked to fix their gaze upon the examiner's index finger tip about 30 cm away As mentioned above, various degrees of intervention of fixation function might largely result in three categories of intensity of percentage reduction in slow-phase velocity (Fig 2), though to some extent, light levels could not be excluded

The multiplication product of amplitude by nystagmus frequency approximates the slow-phase velocity (Bergstedt, 1961, Coats, 1966) When a direct measurement of slow phase velocity is not obtainable, P A F is another simple parameter to be measured It is worth considering, therefore whether or not slow-phase velocity is replaced by P A F as a parameter for evaluating the amount of visual suppression

Using P A F. as a parameter, we estab-

Correlation Between Slow Phase Eye Velocity and P A F

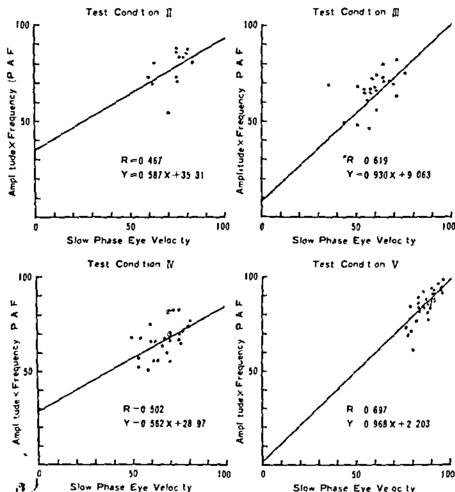


Fig 4 Clear correlation definitely existed between the percentage reduction of suppression in slow phase velocity and that in P A F

lished the percentage reduction of caloric nystagmus induced by eye opening and ocular fixation during the same time course as that of Test Condition II in each test condition (Fig 3) The percentage reduction of P A F also exhibited almost the same value in each test condition without any correlation to water temperature applied as caloric stimulus, which proved significant (Fig 3) On the other hand the amounts of the percentage reduction of P A F were dependent upon test conditions as shown in Fig 3 It was most strongly suppressed in Test Condition V more weakly in Test Conditions II and IV than in Test Condition V and most weakly in Test Condition III This classification also proved significant ($p<0.05$) (Fig 3) As described above great similarity existed between the amounts of percentage reduction of the P A F and those of the slow phase velocity Another important problem is whether or not slow-phase velocity is replaced by P A F in each test condition The mean percentage reduction of caloric nystagmus induced by eye opening and ocular fixation did not depend upon the water temperature applied as caloric stimulus but, on the other hand depended upon the test condition (Figs 2, 3) Therefore, the correlation between the mean percentage reduction in slow-phase velocity and that in P A F was statistically evaluated in each subject under the same test conditions without regard to

water temperature used as caloric stimulus As shown in Fig 4, the amounts of percentage reduction of the P A F were definitely correlated with those of the slow phase velocity In Test Condition II, the level of significance was 5%, i e slightly higher than that in other test conditions This may be due to the small number of samples as data were discarded whose ENG baseline drifted markedly and visual suppression of slow phase velocity and P A F could not be evaluated accurately This sometimes occurred under Test Condition II In the four test conditions the level of significance was 0 05% in both Test Conditions III and V In Test Condition IV 0 5% The amount of suppression in slow phase velocity of caloric nystagmus induced by eye opening and ocular fixation is interchangeable with the amount of suppression in P A F This correlation between the two parameters favours the routine test

ZUSAMMENFASSUNG

Bei 32 normalen Personen wurde der Effekt der offenen Augen und der Fixation auf kalorischem Nystagmus während der maximalen Winkelgeschwindigkeit der langsamen Phase untersucht Die mit offenen Augen und Fixation ausgelöste Suppression war abhängig von der visuellen Kondition aber einerseits nicht von der Temperatur des kalorischen Stimulus Eine andere auffällige experimentelle Tatsache war daß die statistische Wechselbeziehung zwischen der Suppression der langsamen Phase und der mit der Amplitude multiplizierten Frequenz des kalorischen Nystagmus (P A F) während der Periode der offenen Augen und Okularfixation bestand Diese beiden Parameter kann man im visuellen Suppressionstest des kalorischen Nystagmus miteinander an die Stelle setzen Diese experimentelle Tatsache vergrößert den praktischen Wert des visuellen Suppressionstests der kalorischen Nystagmus

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EFFECTS OF CALORIZATIONS AND REPEATED UNIDIRECTIONAL ANGULAR ACCELERATIONS ON THE NYSTAGMUS OF UNILATERALLY LABYRINTHECTOMIZED CATS

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Abstract Right unilateral labyrinthectomy of cats resulted in a left beating spontaneous nystagmus that was still weakly present in most animals when recorded in darkness 2 months after surgery Cats given cold calorizations of the non operated ear during the month following surgery showed no differences in behavior optokinetic nystagmus spontaneous nystagmus or rotation induced nystagmus when compared with operated cats which received no caloric stimulation All operated cats showed a reduction in optokinetic responses that was marked to CCW stimulus and that persisted through second month after surgery Most nystagmic re ses to angular accelerations were bidirectionally minished by more than 50% 1 month after surgery A series of 15 unidirectional habituation trials was ineffective in producing a directional imbalance in the nystagmic responses of operated cats but the series was effective with non operated Control animals It appears that an intact vestibular system may be necessary for directionally specific habituation effects to evidence themselves One month after the habituation series all operated cats showed improved nystagmic output while Control cats showed a trend toward equalization of responses for the two directions

A main consequence of the loss of one labyrinth in mammals is the rapid onset of a spontaneous nystagmus the fast phase of which is directed toward the unoperated side Initially, this nystagmic response can be observed visually Over time as the system adapts, the response can no longer be directly observed, but it can be recorded in total darkness Although aspects of these short and long term oculomotor effects of unilateral labyrinthectomies are understood there is no information available concerning techniques by which the recovery process might be modified or about the course of vestibular habituation in such surgically treated animals Thus the present study was designed to (*a*) investigate the effects of repeated caloric irrigations on the recovery processes of cats that had a unilateral labyrinthectomy, and (*b*) determine the effects of repeated unidirectional angular accelerations on nystagmic responses for the same groups of animals

METHOD

Subjects

Each of 20 cats was randomly assigned to one of four equal groups a (non operated) Control group and three groups that underwent right labyrinthectomies Of the latter, one group was given a set of 15 counterclockwise (CCW) rotations a month after surgery (Op CCW), a second group was given a set of 15 clockwise (CW) accelerations a month after surgery (Op CW), and the third group received first a series of caloric irrigations during the

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month following surgery (an attempt to modify the recovery process) and then a set of 15 CCW angular accelerations (Op-Cal-CCW). Other tests were identical for all animals. Restraint was effected by the method of Henriksson et al (1961). Testing of each animal required a 3-month period. Animals which completed the entire experiment numbered, three in the Control, four in the Op-Cal-CCW, five in the Op-CCW, and three in the Op-CW groups. Since results from the latter two groups were equivalent, they were combined for discussion of results.

Procedure

Qualification tests

All animals were first given Qualification tests for spontaneous nystagmus (recorded in darkness), optokinetic nystagmus, and nystagmic responses to two angular stimuli (one CW, the other CCW). Angular stimulation always involved accelerations of 5°/sec² for 12 sec, 2 min at a constant velocity of 10 rpm, and subthreshold decelerations (0.15°/sec²) in darkness.

Surgery

All animals, except those in the non-operated Control group, had the right labyrinth destroyed surgically, using a retro-auricular atticotomy approach. The microscopic surgeries were performed under general anesthesia induced by sodium pentobarbital.

Operated animals were observed for ataxia while standing and walking, and they were tested for spontaneous and optokinetic nystagmus three times weekly during the period from 1 to 4 weeks following the operation.

Calorization

Animals in the Op-Cal group received a series of five caloric irrigations on each observation day (i.e., three times weekly) during the month following surgery. These irrigations of the non-operated ear (26°C water for 30 sec) provoked a nystagmus that opposed the direction of spontaneous nystagmus produced by surgery.

Habituation

One month following the operations, CW and CCW acceleration tests, identical with those conducted prior to the operations, were again administered (Pre-Habituation tests). These were immediately followed by a series of 15 successive unidirectional angular accelerations (either all CW or all CCW) in the dark, a number sufficient to produce significant nystagmus habituation in normal cats (Collins, 1964, Collins & Updegraff, 1966). For animals in the Control group, the Op-Cal CCW group, and Op-CCW group, habituation trials were CCW accelerations, while those for the Op-CW group were CW accelerations. Two additional acceleration tests (identical to the Pre-Habituation tests) were administered immediately following the habituation series (Post-1 Habituation) and again 1 month later (Post-2 Habituation).

Histology

Upon the completion of the data acquisition all cats were sacrificed by means of intracardiac perfusion, and the temporal bones were removed and processed according to the standard temporal bone preparation procedure.

Recording and Scoring

Nystagmus was recorded on an Offner Type T electroencephalograph (3-sec time constant) from needle electrodes inserted beside the outer ocular canthi. Records were scored in 3 sec intervals from the start of the stimulus to the end of nystagmus for number of beats and amount of slow-phase eye displacement, response durations were also tabulated. Mean total output scores for each of the three measures were calculated for each group. Slow phase measurements were converted to degrees of eye movement by a conversion factor taken from recordings of optokinetic nystagmus obtained prior to each test session.

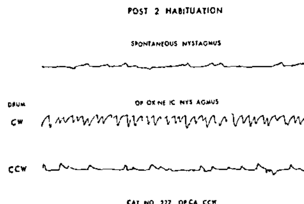


Fig 1 Tracings of typical spontaneous and optokinetic nystagmus from a labyrinthectomized cat 2 months after surgery Spontaneous nystagmus was recorded in darkness Tracings are 22 5 sec in duration

RESULTS AND DISCUSSION

Effects of surgery

All operated animals showed left-beating spontaneous nystagmus following surgery, the intensity of these responses (recorded in darkness) differed considerably among animals

Just prior to habituation trials, 1 month after surgery, all operated animals showed weak [illegible]-beating spontaneous nystagmus Some [illegible] nystagmus was still recordable (but not directly observable in the light) from most of the operated cats 2 months after surgery (Fig 1) Honrubia et al (1971) recorded very low frequency spontaneous nystagmus in the dark from cats 8 months after unilateral labyrinthectomy

One month after surgery, the operated animals were given optokinetic stimulation prior to a series of rotational trials A clear nystagmus reduction from the Qualification test was obtained during CCW rotation of the drum, optokinetic responses to CW drum rotation were reduced to a lesser extent (see Fig 2) *These directional differences in optokinetic* nystagmus were still evident 2 months after surgery Honrubia et al (1971) obtained similar optokinetic nystagmus findings from unilaterally labyrinthectomized cats examined 7 and 14 days after surgery Our Control animals, it should be noted, showed some bidirectional reduction in optokinetic output from the Qualification levels

Prior to the 15 habituation trials, each animal received a CW and a CCW angular acceleration (Pre-Habituation test) Control animals showed some increase in the three measures of nystagmus from the Qualification to the Pre-Habituation test (Table I) The operated animals, on the other hand, showed a marked reduction, from Qualification test levels, of both slow phase nystagmus and the number of eye movements, and a lesser reduction in response duration, for both directions of turning (Table I) However, CW accelerations showed greater reductions than CCW

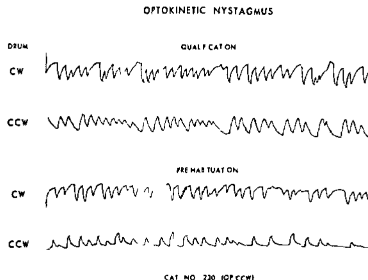


Fig 2 A comparison of tracings of optokinetic nystagmus from a cat before and 1 month after unilateral labyrinthectomy The response of this animal to CCW drum rotation was better than that of any other operated cat in this study Note the more subtle changes in response to CW drum rotation Tracings are 22 5 sec in duration

Table I *Percentages of change in nystagmic output from the Qualification test to the Pre-Habituation test*

The eight Operated cats include five from the OP-CCW and three from the OP-CW groups

Group	N	Measure	% Increase or decrease from Qualification test CW	CCW
Control	3	Slow phase	+13	+31
		No of beats	+ 5	+33
		Duration	+11	+19
Operated (plus caloric irrigations)	4	Slow phase	−75	−57
		No of beats	−63	−38
		Duration	−56	−19
Operated	8	Slow phase	−64	−55
		No of beats	−67	−54
		Duration	−41	−31

stimulation The bidirectional reduction in the output measures of rotation induced nystag mus from these operated animals generally exceeded 50% (8 of 12 mean scores) and were thus close to the values obtained from cats with one plugged horizontal canal (reductions of about 50%) both for response duration as measured by Money et al (1965) and for "system gain" as measured by Zuckerman (1967)

Effects of calorization

There was no evidence from the visual observations of spontaneous nystagmus and ataxic gait and posture, nor from the recordings of spontaneous and optokinetic nystagmus that the animals which were given caloric irrigations during the month following surgery differed in recovery time or other characteristics from those operated animals which were not so stimulated The Pre-Habituation rotation tests also showed no apparent effect of the calorizations This latter finding agrees with previous studies of normal cats (Collins, 1964) which showed no significant transfer of the effects of repeated caloric stimulation on responses to angular accelerations

Effects of the habituation series

The effects of the 15 unidirectional rotational habituation trials were assessed in the Post-1-Habituation test which immediately followed the habituation series The Control animals showed the expected results (e g , Collins & Updegraff, 1966), namely a clear reduction in duration number of beats, and slow-phase nystagmus that was relatively specific to the direction of eye movement repeatedly elicited (Table II)

Table II *Percentages of change of nystagmic output from the Pre-Habituation test to the Post-Habituation tests*

Scores are presented for responses in the direction repeatedly elicited (Hab) and in the direction not elicited (Non Hab) during the habituation series

Group	N	Measure	% Increase or decrease from Pre Habituation to Post 1 Habituation: Non Hab	Hab	% Increase or decrease from Pre-Habituation to Post 2 Habituation: Non Hab	Hab
Control	3	Slow phase	−31	−65	−43	−48
		No of beats	10	−50	−21	−41
		Duration	−19	−38	−26	−31
Operated (plus caloric irrigations)	4	Slow phase	+10	− 9	+31	+23
		No of beats	0	− 4	0	+20
		Duration	+20	− 4	0	−12
Operated	8	Slow phase	−19	−23	+21	+17
		No of beats	− 7	−18	+21	−12
		Duration	−11	0	− 6	0

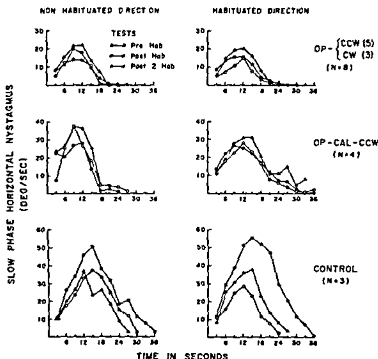


Fig 3 Slow phase nystagmus plotted in 3 sec intervals for angular acceleration conducted immediately before immediately after and 1 month after a series of 15 successive unidirectional habituation trials Control cats and Op-Cal cats received CCW angular accelerations as habituation stimuli, in the Op group five cats received CCW and three cats received CW habituation stimuli

However, neither the calorized nor the non-calorized operated group showed any directionally specific effect of the habituation series (Fig 3) Indeed, there was almost no discernible effect of the 15 rotations on the calorized group and only slight bidirectional reduc- ns (around 20% each) for the non-calorized up of surgically treated cats (Table II) It appears that an intact vestibular system may be necessary for directionally specific habituation effects to evidence themselves

Recovery

One month after the habituation series, all ani mals were retested (Post-2-Habituation) Compared to Post 1-Habituation, Control cats showed slight overall recovery perhaps better defined as a trend toward equalization of all three measures of response in the two directions, i e , output in the habituated direction increased and output in the unhabituated direction declined somewhat (Fig 3) Operated animals in all cases had increased slow-phase output for both directions in Post 2 Habitua tion tests (as compared with Post-1) and in fact, had somewhat greater slow phase output than during the Pre-Habituation test (but were still well below the slow phase level demo strated in the preoperative Qualificatio session) For the operated cats, response dur tions changed insignificantly while the numbe of eye movements showed recovery in on direction for the calorized groups, and in th other direction for the non calorized groups

It would appear that recovery from a un directional habituation series may differ i character between normal cats and cats un laterally labyrinthectomized Since th habituation series had little influence on th operated animals, their response patterns ma reflect almost solely recovery from the surg cal procedure

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Labyrinthectomy was properly placed in th right ears of all operated cats Left labyrinth in these animals were intact microscopicall All ears in Control cats showed normal laby rinthine end organs

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Bei Katzen führte die rechte einseitige Labyrinthektomie zu einem linksschlagenden Spontannystagmus In den meisten Katzen war dieser noch nach zwei Monaten schwach vorhanden wenn im Dunkeln untersucht wurde Die Katzen die kalte Kalorisationen auf das nicht operierte Ohr bekommen hatten innerhalb eines Monates nach der Operation zeigten keine Unterschiede weder im Verhalten optokinetischen Nystagmus Spontannystagmus noch im verursachten angeregten Drehnystagmus verglichen mit operierten Katzen die jedoch keine kalte Kalorisation erhielten Alle operierten Katzen zeigten eine Reaktionsverminderung des optokinetischen Nystagmus welcher sich nach linkem (dem Uhrzeigersinn entgegen) Umdrehungsreiz mehr bemerkbar machte und auch noch wahrend des zweiten Monates nach der Operation vorhanden war Einen Monat nach der Operation waren die meisten nystagmischen Reaktionen auf Winkelbeschleunigung um mehr als 50% nach beiden Seiten hin vermindert Eine Serie von 15 Untersuchungen die Gewohnheit der Katzen nach einer Richtung hin zu erproben, also einen seitlichen Ungleichgewichtszustand der nystagmischen Reaktion hervorzurufen war in operierten Katzen erfolglos dies war jedoch erfolgreich bei den nichtoperierten Kontrolltieren Es ist wahrscheinlich, daß ein unbeschadigtes vestibulares System notwendig ist ehe sich besondere seitliche Gewohnheitswirkungen bemerkbar machen Einen Monat nach der Gewohnheitsserie wie oben angeführt zeigten alle operierten Katzen im Gegensatz zu den Kontrollkatzen eine verbesserte nystagmische Leistung die Kontrollkatzen zeigten nur eine geringe Reaktionsausgleichung nach beiden Seiten hin

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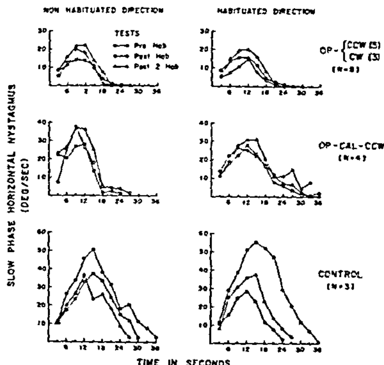


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SEMICIRCULAR CANAL FUNCTIONAL ANATOMY IN CAT, GUINEA PIG AND MAN

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Abstract Formulation of semicircular canal transfer functions have to date been restricted to idealized cases Recent information in cat guinea pig and man on the precise orientation of the semicircular canals allows a more realistic specification of the forces acting on the cupulae during any head rotation

A number of authors have analysed the relationship between head angular acceleration and the forces acting on the cupula, usually treating the canal in an idealized fashion, assuming that the plane of the stimulating [illegible] coincides exactly with the plane the semicircular canal (e g Melvill Jones, 1972, Dohlman, 1935, van Egmond et al, 1949, Valentinuzzi, 1967, Jones & Spells, 1963, Oman & Young, 1972, Fernandez & Valentinuzzi, 1968) Because the semicircular canals are bilaterally symmetrical any angular acceleration must stimulate canals in both labyrinths and depending on the plane of this acceleration relative to the head, more than one canal in each labyrinth could be stimulated The input to central vestibular neurons thus will depend on the plane of angular acceleration, the planes of the semicircular canals in each labyrinth and the extent to which contralaterally matched canals ("synergists") are parallel Our investigations of the semicircular canals in the cat, guinea pig and man show that in most cases the canal planes are not orthogonal within one labyrinth, nor are contralateral synergists parallel (Blanks et al, 1972, 1975*a*, Curthoys et al, 1975*a*) In this paper we examine the effect these departures from an idealized system have for the pattern of canal stimulation

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METHODS

The methods have been described in detail in previous papers (Blanks et al, 1972, 1975*a*, Curthoys et al, 1975*a*) Briefly in dried skulls of cats, guinea pigs and humans the osseous semicircular canals were exposed usually by burring away the lateral most portion of bone overlying the canals Skulls were then placed in a stereotaxic apparatus appropriate for its species and the stereotaxic coordinates of a number of points along each canal were

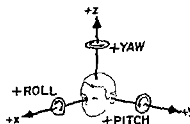


Fig 1 The spatial conventions of the Hixson Niven and Correia system

Table I *Values of coefficients* (A, B, C, D) *of th* *formulae for the canal planes* (Ax+B, +Cz+D=0) *in stereotaxic space*

	Coefficients				e of centre			Distance between contralateral centres in mm
	A	B	C	D		›	Z	
Cat								
Left horizontal	+0 393	+0 112	−1 000	+ 3 302		14 34	+2 54	28 68 (28 2)
Left anterior	+0 790	−1 000	+0 335	+14 875	[illegible]	12 44	+4 52	24 88 (25 8)
Left posterior	+1 000	+0 914	+0 605	− 5 655		+12 38	+3 18	24 76 (25 8)
Right horizontal	+0 393	−0 112	−1 000	+ 3 302	[illegible]	14 34	+2 54	28 68 (28 2)
Right anterior	+0 790	+1 000	+0 335	+14 875	[illegible]	−12 44	+4 52	24 88 (25 8)
Right posterior	+1 000	−0 914	+0 605	− 5 655	[illegible]	12 38	+3 18	24 76 (25 8)
Guinea pig								
Left horizontal	+0 991	−0 345	−0 885	+ 6 307	−2 93	+ 7 02	+1 06	14 04
Left anterior	+0 729	−1 000	+0 210	+ 8 677	−3 30	+ 6 67	+2 10	13 34
Left posterior	+1 000	+0 486	+0 650	+ 1 684	4 53	+ 6 00	−0 03	12 00
Right horizontal	+0 991	+0 345	−0 885	+ 6 307	2 93	− 7 02	+1 06	14 04
Right anterior	+0 729	+1 000	+0 210	+ 8 677	−3 30	− 6 67	+2 10	13 34
Right posterior	+1 000	−0 486	+0 650	+ 1 684	−4 53	− 6 00	−0 03	12 00
Human								
Left horizontal	+0 375	−0 169	−1 000	+15 292	−4 06	+37 04	+7 50	74 08
Left anterior	+0 856	−0 987	−0 004	+37 751	−3 75	+34 47	+9 66	68 94
Left posterior	+1 000	+0 732	+0 398	−19 997	−7 37	+34 61	+5 52	69 22
Right horizontal	+0 375	+0 169	−1 000	+15 292	−4 06	−37 04	+7 50	74 08
Right anterior	+0 856	+0 987	−0 004	+37 751	−3 75	−34 47	+9 66	68 94
Right posterior	+1 000	−0 732	+0 398	−19 997	−7 37	34 61	+5 52	69 22

measured by means of a fine probe attached to a micromanipulator

The conventions defining the stereotaxic space of cat, human and guinea pig are vari able we chose to adopt the widely accepted Hixson, Niven & Correia system (1966) and to transform the stereotaxic conventions for the different species into that system which is shown schematically in Fig 1 In this system the x axis is the naso-occipital axis (anterior positive), the y axis is the interaural axis (left ear positive) and the z axis is a vertical axis (dorsal positive) Yaw in a positive direction is counterclockwise (or to the left), roll in a positive direction is right ear down, and pitch in a positive direction is nose down

A multiple regression program fitted a plane to the points for each canal by a least-squares technique This program provides the magnitudes and signs of all the coefficients of the formula of a plane in three dimensions whose generalized equation is $Ax+By+Cz+D=0$ (Bers 1969) In addition the raw data was also processed by principal component analysis program the eigenvectors corresponding to the smallest eigenvalue are the direction cosines of the plane best fitting the data points (i e the eigenvectors are the normalized coefficients A, B and C in the formula given above (Thurstone, 1957) This latter technique returns no D term meaning that the coefficients are for a plane passing through the stereotaxic origin (0,0,0) *parallel* to the plane of the canal Against this deficiency however is the advantage that the principal component procedure fits a plane to the data points by minimizing the perpendicular deviations from the plane, whereas the least-squares technique minimizes vertical deviations from the plane Although the results of the two techniques are almost identical, because of the former advantage we have in previous papers used the data from the principal component analysis in all planar calculations Here we present the

Table II *Angles between canal planes and an earth horizontal plane of angular acceleration when the horizontal canal is positioned to be optimally stimulated, either unilaterally or bilaterally*

Angle between an earth horizontal plane of angular acceleration and the plane of the	Unilateral optimal orientation			Bilateral optimal orientation		
	Angle	Cosine	Attenuation (dB)	Angle	Cosine	Attenuation (dB)
Cat						
Horizontal canal	0°	1 0	0	6°	0 99	−0 02
Anterior canal	89 62°	0 01	−21 78	86°	0 07	−11 56
Posterior canal	94 23°	0 07	−11 32	82°	0 14	− 8 56
Guinea pig						
Horizontal canal	0°	1 0	0	15°	0 97	−0 15
Anterior canal	122 15°	0 53	−2 74	71 19°	0 32	−4 92
Posterior canal	82 36°	0 13	−8 76	75 91°	0 24	−6 14
Human						
Horizontal canal	0°	1 0	0	9°	0 99	− 0 05
Anterior canal	111 76°	0 37	−4 31	75°	0 26	− 5 87
Posterior canal	95 75°	0 01	−9 99	90 8°	0 01	−18 55

equations of the actual semicircular canal planes using the coefficients from the least-squares technique (Table I) These coefficients were obtained by averaging the absolute values of the coefficients for the right and left labyrinths, and then determining the signs of the coefficients on the basis of the orientation of the canal plane and the side of the head which it belonged Signs of all the coefficients in some equations have been reflected relative to some previously published equations in order to maintain comparable co-ordinate conventions for all three species The canal plane coefficients produced by both fitting procedures were then processed by another program which yielded all the angles between canals, between each canal and every stereotaxic plane, as well as angles for positioning the skull for optimal rotatory stimulation of a given semicircular canal The latter angles were used in another program (called ROTA) to rotate the original data points for each canal and print them so that the plane of the computer printout corresponded exactly to the plane of the canal This projection was considerably magnified (cat and guinea pig 51 5×, human 25 5×) enabling accurate measurement of the radii of curvature (*R*) of all canals in a skull and determination of the stereotaxic coordinates of the centre of the circle of which each canal was an arc Finally the coordinates of centre were rotated back to the original stereotaxic coordinates

RESULTS

The mean values of the coordinates of the centre for each canal for each species are presented in Table I These coordinates allow precise positioning of the centre of a given canal over the axis of rotation of an angular acceleration device to minimize linear acceleration during rotary stimulation Because the raw data points were obtained from the medial-most wall of the osseous canal, the ROTA technique requires corrections if estimates of *R* of the membranous ducts are needed As far as the coordinates of canal centres are concerned, no corrections are needed the membranous duct adheres to the outermost wall of the osseous canal (Johnsson, 1971, Gray, 1907, Gray, 1951, Curthoys et al , 1975*b*) so any variation in the position

Table III *Coefficient of variation of angles between canal planes for the three species*

Angle between the plane of the	and the plane of the	Cat	Guinea pig	Man
Horizontal canal	anterior canal	0 01	0 05	0 07
Anterior canal	posterior canal	0 04	0 07	0 05
Horizontal canal	posterior canal	0 04	0 06	0 05
Left anterior canal	right posterior canal	0 29	0 14	0 29
Left posterior canal	right anterior canal	0 31	0 13	0 28
Left horizontal canal	right horizontal canal	0 74	0 33	0 75

of the membranous duct defines concentric circles with the same centre

The distance between contralateral canal centres (right columns in Table I) can be used to provide an estimate of the centrifugal force acting on a given canal (or the utricles) during constant velocity stimulation when the centre of the interaural axis is positioned over the axis of rotation The distances between canal centres for the cat can be compared with measures from Fernandez & Valentinuzzi (1968) which are given in italics after each distance, the largest discrepancy is only of the order of 1 mm (between posterior canals)

The angles between the canals in each cat labyrinth are very close to being mutually perpendicular, whereas for the human and the guinea pig the angles between ipsilateral canals deviate considerably from being mutually perpendicular So for guinea pig and man if the horizontal canal in one labyrinth is oriented to be exactly in the plane of angular acceleration stimulation then the other canals in that labyrinth will receive some attenuated level of stimulation Assuming that the magnitude of angular acceleration stimulation is attenuated in a cosine fashion as a canal is tipped out of the plane of stimulation it is possible to specify the magnitude of the stimulation actually presented to the other canals (The assumption is not only reasonable geometrically but has also been confirmed by the demonstration that the magnitude of the physiological response of cat canicular primary afferent neurons decreases as a cosine function as the canal is tipped out of the plane of angular acceleration (Blanks et al , 1975*b*)) Attenuation of stimulus level can be given either directly in terms of the cosine of the angle between the canal plane and the plane of acceleration or in terms of decibels below the actual stimulus level, simply by multiplying the logarithm of the absolute value of the cosine by 10 Table II presents for the three species the angles between canals, the cosines and the attenuation in dB, for two general cases firstly when the horizontal canal in one labyrinth only is exactly positioned in the plane of angular acceleration (Unilateral Optimal Orientation) and secondly when the skull is positioned to optimally stimulate the horizontal canals in both labyrinths (Bilateral Optimal Orientation) Since contralateral horizontal canals are not parallel in any of the species the latter position necessitates some compromise (see Table II) For both positions there is notably less spread of stimulation for the cat compared to both man or the guinea pig reflecting the fact that cat ipsilateral canals are very close to being mutually orthogonal More spread occurs between the ipsilateral horizontal and anterior canals in the guinea pig where even if the horizontal canal were oriented to be exactly in the plane of stimulation the level of acceleration stimulating the ipsilateral anterior canal would only be reduced by about half Similarly, the human anterior canal will receive about 0 37 (−4 31 dB) of the level of acceleration delivered exactly in the plane of the ipsilateral horizontal canal In short the angular structure of the semicircular canals in the guinea pig and man

means that there is always considerable over lap of horizontal and anterior canal stimulation with any initial head orientation

Within each species the canals are not parallel with their contralateral synergists The departure from parallelism is quite marked in man and guinea pig being of the order of 20° and 30° respectively Taken on average the horizontal canals of the guinea pig and human are tipped down laterally whereas the cat horizontal canals are tipped up laterally However the angles between contralateral synergist canals particularly contralateral horizontal canals shows wide variability An index of variability corrected for the magnitude of the scores from which it is derived is the coefficient of variation—the standard deviation divided by the mean Table III presents the coefficient of variation for angles between canal planes for the three species For all species the angles between canals in a labyrinth show uniformly small variability coefficients of variation of the order of 0 05 Angles between contralateral vertical canals are much more variable and the angle between contralateral horizontal canals the most variable of all Practically this ans that in some human skulls the hori ontal canal planes on the right and left sides of the head angle upward rather than down ward as the average do

DISCUSSION

Validation of the measurement technique

The strongest validation of this technique for measuring the planes of semicircular canals has come from physiological studies which have identified the optimal plane of angular acceleration stimulation of primary neurons of each canal in the cat and compared these planes with the planes determined by these anatomical means (Blanks 1973 Estes et al 1975) The discrepancies average about 6° One explanation for these slight differences is that in the living preparation the thickness of tissue overlying the infraorbital ridges might cause a slight pitching of the head as compared to when the skull alone is fixed in the stereotaxic frame Another explanation might be slight differences in stereotaxic instruments We have in fact observed such differences be tween instruments from both the same and dif ferent manufacturers (Blanks et al 1972 Estes et al 1975) A third explanation is that the endolymph fluid forces acting to produce the physiological data include the endolymph in the utricle as well as in the semicircular canals That portion of the endolymph toroid passing through the utricle may be deflected or limited by the utricular walls such that a single plane could not be fitted to the entire endo lymph ring However rather than explaining the minor differences between the anatomical and physiological studies we should empha size how close the results from these two types of studies really are

Fernandez & Valentinuzzi s projection tech nique (1968) does not provide all angles neces sary to specify the orientations of the canals —thus they specified the cat horizontal canal to be in the horizontal plane and this error (the horizontal canal is actually about 21° above the horizontal plane open anteriorly) has already appeared in the literature (Boud reau & Tsuchitani 1973) Also it seems this technique makes the assumption that the vertical canals are perpendicular to the hori zontal plane rather than measuring this angle Our measurements show fairly substantial departures from perpendicularity to the hori zontal plane in some cases in the cat and the guinea pig the anterior canals are at 105° and 100° and the posterior canals 66° and 60° to the horizontal plane respectively

Labyrinth orientation in the skull

Different species exhibit different orientations of the whole labyrinth within the skull Mayne (1965) and Jones & Spells (1963) have dis cussed the match of the size of the semi circular canals of animals of different species to the natural frequencies of head movements of the species We consider that it may be

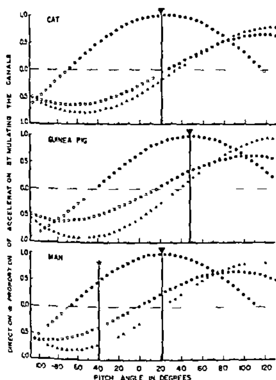


Fig 2 The directions and proportions of an earth horizontal anticlockwise angular acceleration stimulus actually stimulating each canal in a left labyrinth for cat guinea pig and man as the head is pitched around the *interaural* axis The pitch angle has been arbitrarily referred in each case to the stereotaxic horizontal (0°) nose up is negative Circles=horizontal canal squares=anterior canal, triangles=posterior canal The closed symbols denote that the stimulus is in an excitatory direction for the canal open symbols that the direction is "inhibitory

possible to extend this concept of "matching" to the relationship between semicircular canal orientation and the "natural" position of the head Perez (1922), Girard (1923) and Lebedkin (1924) put forward the hypothesis that the horizontal canal plane approximates the natural (horizontal) position of the head Girard pointed to the horizontal canal planes of bison and sheep as being two animals with entirely different natural head positions, and having entirely different horizontal canal planes relative to the bulk of the skull Girard in fact proposed using the plane of the horizontal canal as a skull standardization plane (as opposed to e g the Reid Line) This proposal has been followed by French craniometrists (Fenart, 1967, Fenart & Dardenne 1968 Fenart & Destombes, 1972, Fenart & Heim 1968 Fenart et al , 1966)

Vectorial resolution by the semicircular canals

In normal head movements the brain must be supplied with information about the magnitude direction and orientation of the angular acceleration How this occurs is essentially the problem of vectorial resolution originally discussed by Summers et al (1943) The problem lies in the fact that many angular accelerations of different magnitudes in different planes can each produce a given firing rate in an afferent neuron from one canal Firing rates of neurons from one canal alone *cannot signal both the magnitude and the plane* of the angular acceleration Only relative amounts of activity of neurons from different canals can resolve both magnitude and plane of acceleration

Any angular acceleration of the head causes a unique pattern of activation of the six semicircular canals in the head Exactly what this pattern is, will be determined by the orientation of the canals in the head and their relationship to the plane of the angular acceleration Usually the plane of head rotation will not correspond exactly to the plane of a canal, which is in any case not parallel with its contralateral synergist and so all six canals will receive some level of stimulation Since we have specified the planes of the canals in the skull and since it is known that ampullopetal endolymph "flow" is excitatory for horizontal canal receptors but "inhibitory" for vertical canal receptors it is possible to specify exactly the pattern of angular acceleration stimulation in terms of the relative magnitudes and (excitatory) directions presented to, or incident upon, the entire bilateral semicircular canal system during any head rotation

Fig 2 shows for the three species the pro-

portions of an earth horizontal angular acceleration stimulating all three canals in a labyrinth as a head, positioned for optimal bilateral horizontal canal stimulation, is pitched around the interaural axis These curves show, for the three canals in a left labyrinth during an anticlockwise earth horizontal angular acceleration, both the proportion of acceleration actually stimulating each canal and whether the stimulation is in an excitatory (closed symbols) or "inhibitory' (open symbols) direction for that canal The direction of endolymph flow known to be facilitatory for a given canal has been taken into account in plotting these curves So consider for example a human subject whose head is fixed in the centre of a turntable and is positioned 40° in a ' nose up ' (negative) pitch position If he is stimulated by an earth horizontal angular acceleration in an anticlockwise direction of magnitude X then his left horizontal canal will be stimulated in an excitatory direction by about 0 45X, his left anterior canal will be stimulated in an inhibitory direction by about 0 40X, and his left posterior canal will be stimulated in an inhibitory direction by about 0 75X (see ver- l line with star in Fig 2) The input to the ht labyrinth will be an identical pattern but he excitatory dimension will be reversed This particular plane of angular acceleration thus provides a unique pattern of stimulation to the six semicircular canals As the head is pitched away from this position the pattern changes according to the curves shown in Fig 2 Reversing the direction of angular acceleration also reverses the excitatory dimension

The differences in the curves between the species reflect differences in stereotaxic conventions and differences in the angular relationships amongst the canals and between the canals and the stereotaxic planes The arrows in Fig 2 show the optimal pitch angle for bilateral stimulation of both horizontal canals The proportions and directions of acceleration affecting the other canals at this position can be read off and correspond to the values for Bilateral Optimal Orientation presented in Table II The formulae presented in Table I (and elsewhere) enable specification of the unique pattern of stimulation incident on the canals for variations in roll and/or for stimulation planes other than an earth horizontal one

The physical differences which exist between canals (Curthoys et al , 1975*b c*) imply that (after transduction) the relative proportions of neural activity will differ from the stimulus proportions shown here So whilst most of the dimensions of the membraneous ducts and ampullae are not detectably different for the three canals in a labyrinth within each species (Curthoys et al , 1975*b*) there does seem to be a trend for the anterior canal to have a larger R than the posterior and horizontal canals (Curthoys et al , 1975*c*) In order to represent the relative proportions of neural activity after transduction the amplitudes of the cosine functions in Fig 2 probably should be multiplied by the relative R of the canals for the species

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ZUSAMMENFASSUNG

Die Formulierungen von Bogengangen Übertragungsfunktionen waren bisher auf ideale Fälle beschrankt Neue Kenntnisse uber die genaue Lage (Orientierung) der Bogengange und ihre relativen Größen erlauben es eine realistischere Angabe der Krafte die auf die Cupula wahrend jeder einzelnen Kopfrotation wirken anzugeben

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MENIERE'S DISEASE

A Neuropsychological Study

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Abstract A neuro-psychological study is described comprising 19 patients with Meniere s Disease who were participating in an experimental lithium therapy programme The patients evidenced an organic dysfunction which is not entirely peripheral The interpretation of the results are not incompatible with an attempt to reconcile neuro-psychological and psycho-somatic viewpoints regarding etiological aspects of Meniere s Disease An evaluation of the lithium effect was performed

Psychological aspects of Meniere's Disease (MD) have been considered by several investigators, who have applied personality tests rating scales in the psychological assessment of these patients (e g Sirala et al , 1965, Hinchcliffe, 1976, Watson et al , 1967, Williamsen & Giffort, 1971, Stephens, 1975) Only one previous study of MD patients has utilized a neuro psychological test battery Lochen (1970) compared 30 MD patients with 50 patients with severe central cerebral atrophy with regard to an extensive set of parameters, among those the outcome of neurological examination including Pneumoencephalogram (PEG) and Electroencephalogram (EEG) and the results of a varied battery of neuropsychological tests All the MD patients showed pathological neurological and/or pneumoencephalographic findings indicating organic brain damage

This impression was supported by the fact that the MD group and the control group had very similar patterns in their test scores

The present investigation describes a group of MD patients, who were available for study because of their participation in an experimental lithium therapy programme (Thomsen et al , 1974) A preliminary report of the neuropsychological findings was given by Zilstorff et al (1975) The study is exploratory in view of the fact that no other control material was available than the normative data of the tests

METHOD

Subjects

The subjects were 19 MD patients, 9 males and 10 females The mean age was 53 2 years (range 35–70 years) Twelve were admitted to the hospital for 4 days for the purpose of the study, 7 were tested as out patients At the time of testing all subjects had a relatively low socio-economic level On a scale from 0–7 they obtained a mean score of 0 84 (Svalastoga, modified scale, 1959) The range was from 0 to 4 Eighteen of the 19 subjects had scores on levels 0–2

In order to investigate whether the localization of the hearing and vestibular impairment have any relationship to a lateralization of a possible organic dysfunction, the patients were divided into those with mainly left and those with mainly right hearing defects There were 9 subjects in each group since one was

excluded on account of ambidexterity There was no statistically significant difference between mean age and social class in the two groups

Test battery

In selecting the tests it was considered that the results should give quantitative data which could be treated by statistical methods In addition we wanted to study a wide range of cognitive functions These were learning, memory, abstraction, activation and Goal Directed behaviour We also found it important to estimate the hand preference and the premorbid intelligence level To clarify the question of lateralization of the dysfunction easy-to-verbalize (verbal) tests and hard to-verbalize (non verbal) tests, presumably representing functions in left vs right hemisphere in right-handed persons, were also included

The test battery was as follows

Function

Learning and long term memory
Short term memory
Abstraction
Activation and Goal Directed behaviour

Verbal

Paired associates
Sentence reproduction
Similarities
Word mobilization
"100–7"
GDSA (Goal Directed Serial Alternation)

Non verbal

Ruth Andersen Visual Gestalts (RA)
Finger Maze
Memory for Designs
Block Design
Bourdon

Description of Tests

Paired Associates The subject is asked to read aloud a list of five meaningfully and five arbitrarily associated nouns He was instructed that subsequently he should respond with the associated item when the first half of each pair was presented After four presentations of the list the checking took place If he did not succeed in a correct reproduction he was asked to read the word pairs twice more and was then checked again The test continued in this way either until the pairs were learned or until the subject had read the series ten times From previous clinical experience it was expected that the pairs would be learned within six readings After a pause of approximately 60 minutes the subject was checked again and the expected result was a correct reproduction of at least eight of the ten pairs

Visual Gestalts (Ruth Andersen 1968) The material to be learned consists of four complex designs each circumscribed by well known geometrical figures a circle a square a triangle and a semicircle Each gestalt is easily differentiated into subgestalts The subject is given a pencil and a sheet of paper printed with the circumscribed figure After a 10 sec exposure of the model the subject draws as much as he can remember If he makes errors or can not proceed further he is given a new sheet of paper with the circumscribed figure into which the experimenter copies the subgestalts already reproduced correctly The model is then presented for another 10 sec period When he has succeeded in reproducing the figure correctly he continues with the next figure

An hour after the RA examination the subject was again given a sheet with the outlined figure and without seeing the model he had to draw as much as he could remember If there were omissions or errors the experimenter added a subgestalt on the next piece of paper and sheet by sheet the subject was provided with an increasing amount of clues to prompt his memory for the rest of the pattern

Finger Maze The subject was given a tactual finger maze shielded from his view The criterion of learning was three correct trials in succession

Sentence Reproduction Twelve sentences of different length (three each of 14 20 22 and 28 syllables) were read aloud to the subject after each sentence he was asked to repeat it

Memory for Designs (MFD Graham & Kendall 1960) The test consisted of fifteen designs Each design was shown for 5 sec then it was removed and the subject was asked to draw the figure on a piece of paper The kinds of error scored are for example omissions reversals or loss of whole configuration The errors were weighted on a scale from 0–3

Similarities Block Designs and Vocabulary are all subtests from WAIS (Wechsler 1955)

Word Mobilization The subject is asked to mention all the words alternately starting with an "s and a d —as quickly as he is able to within one minute

100 7 The subject is asked to subtract 7 from 100 and continue until he reaches a number near zero Time and number of errors were measured

Goal Directed Serial Alternation (GDSA Melger et al 1970) Here the subject starts with 110 subtracts 7 adds 1 subtracts 7 adds 2 subtracts 7 adds 3 and repeats this alternate subtraction and addition until he reaches a number between 46 and 54 The score is a function of time + errors

Bourdon The test consists of a long page with lines of 60 letters each The subject is requested to cancel the

Table I *MD patients' mean scores in the various tests*
+ age related

Test	Score	Norms
Visual Gestalt		
Learning (number of errors)	4 0	50 Percentiles 2+ } Andersen 1968
Reproduction (number of errors)	15 6	50 Percentiles 6+ } Andersen 1968
MFD (number of errors)	3 0	3 63+ (Graham & Kendall 1960)
Similarities (scaled score)	12 3 (13 5)+	12 1+ } (Hess 1973)
Vocabulary (scaled score)	10 3 (12 2)+	10 4 } (Hess 1973)
Block designs (scaled score)	11 7 (11 3)+	12 3+ } (Hess 1973)
100−7 (number of seconds)	64 3	<60
100−7 (number of errors)	1 5	≤1
GDSA score	229 0	130 4
Bourdon (percent of errors)	34 0	
Word mobilization (number of words)	13 3	

		N
10 word pairs	Learned	6
	Not learned	13
Reproduction of word pairs	≥two less than in learning	5
	<two less than in learning	13
Sentence reproduction	≤all sentences of syllables are reproduced correctly	16
	all sentences of >20 are reproduced correctly	3
Maze learning	≤five trials before the maze is learned	11
	>five trials before the maze is learned	7

letters a b n and p for 10 minutes Omissions and incorrectly crossed letters are counted as errors The error percentage is calculated in terms of the number of letters attempted

Hand preference test (Annett 1970) The subject is asked twelve questions about his hand preference in different activities

In addition the *Vocabulary test* from the WAIS was applied in order to estimate the premorbid intelligence level

The psychological examination was part of a closed double blind cross-over study with 6 months lithium treatment and 6 months placebo treatment No other treatment was given Serum lithium concentration was checked every 2 weeks When they were treated with lithium levels were kept within the therapeutic range (0 7–1 0 mmol/l)

The interval between the two testings was about half a year We attempted to examine the subjects once under lithium treatment and once under the placebo condition Nineteen patients were tested once of these we only succeeded in retesting nine Of the 9 patients 4 were females The mean age was 52.2 years with a range 37–64 The mean social class was 0 89 Five of the patients were placed on level 0 and 4 on level two

RESULTS

1 *The total group of patients with Meniere s Disease*

Where it was possible the patients' scor were compared with norms obtained fro age related normal groups in view of the at sence of controls In some cases such norm were not available

In Table I the group's mean scores on th tests are shown along with the norm-derive expected scores, some of which are age related Visual Gestalt, MFD and subtests from WAIS Most remarkably is a high score on the reproduction of Visual Gestalts which indicates a defective long term memory of non verbal material The high scores on GDSA and Bourdon also deserves attention To complete the 100−7 test the patients used



Fig 1 Visual Gestalts A model with its subgestalts

more time than the average population, but more remarkably, also more time than age corresponding group of manic depressive patients in their depressive phase (Heshe et al 1976) The time for this group was only 56 4 sec, while the MD group used 64 3 sec

These results indicate concentration difficulties in MD patients Furthermore it is noteworthy that many of these patients are not able to learn ten Paired Associates, even after ten readings

2 Localization results

As mentioned previously the MD group was divided into those with left and those with right hearing defects All subjects in this analysis were right handed (Annett, 1970) Significance was tested by two-tailed Mann Whitney U Test and Fisher Exact Probability Test

As can be seen in Table II, patients with a hearing defect in the left ear surprisingly manage worse than patients with defect in the right ear The differences in Similarities and in the reproduction of Visual Gestalts are significant The left ear group also shows a trend of having more difficulties in learning the mazes (p=0 056)

3 Evaluation of the lithium effect

The effect of the lithium treatment on the test results was examined As mentioned before only 9 patients were included in these analyses Wilcoxon matched pairs Signed Ranks test and the Binomial test were applied for the statistical analyses They were used as one tailed tests, as the hypothesis was, that lithium treatment would show a good effect in MD patients

In general, patients with lithium treatment perform more poorly than they do without this kind of drug but only one result reaches the level of significance They commit more errors in learning visual spatial material (p=0 025) However the increased error number is still within normal limits (see Table III)

DISCUSSION

On the basis of this preliminary investigation we are in no position to interpret the findings in an explanatory manner The number of subjects is rather small therefore cautions have to

Table II *Comparison between Morbus Meniere patients where the hearing impairment is located mainly to respectively the right and the left ear (mean values)*

Test	Hearing impairment Left ear (N 9)	Hearing impairment Right ear (N=9)	p
R A Learning(numberoferrors)	5 3	2 5	NS*
R A Reproduction (number of errors)	22 3	9 4	0 004
MFD (number of errors)	2 7	3 6	NS
Similarities (scaled score)	10 7	13 7	0 05
Block designs (scaled score)	9 7	9 9	NS
Vocabulary (scaled score)	11 7	11 6	NS
100−7 (number of seconds)	67 9	63 1	NS
100−7 (number of errors)	1 6	1 6	NS
GDSA score	225 0	225 3	NS
Bourdon (percent of errors)	24 4	34 5	NS
Word mobilization (number of words)	11 6	15 3	NS
The numbers given here are number of subjects			
Ten word pairs			
Learned	1	5	
Not learned	8	4	0 12*
Reproduction of word pairs			
≥Two less than in learning	4	1	
<Two less than in learning	5	8	NS
Maze learning			
≥Seven trials before the maze were learned	4	7	
>Seven trials before the maze is learned	5	(?)	0 056
Sentence reproduction			
All sentences of ≤20 syllables were reproduced correctly	7	9	
All sentences of >20 syllables were reproduced correctly	2		NS

Table III *Comparison between subjects with and without lithium treatment*

Test	With lithium	Without lithium	p
Visual Gestalt			
Learning (number of errors)	4 4	2 7	0 025[a]
Reproduction (number of errors)	12 3	12 4	NS
MFD (number of errors)	2 3	1 6	NS
Similarities (scaled score)	13 0	13 9	NS
Block designs (scaled score)	9 6	11 4	NS
Vocabulary (scaled score)	12 0	12 4	NS
100−7 (number of seconds)	50 3	45 9	NS
100−7 (number of errors)	0 5	0 9	NS
GDSA score	178 7	186 0	NS
Bourdon (percent of errors)	33 2	36 7	NS
Word mobilization (number of words)	14 3	13 7	NS
The numbers given here are numbers of subjects			
10 word pairs			
Learned	4	7	
Not learned	5	2	NS[b]
Reproduction of word pairs			
≥Two less than in learning	1	1	
<Two less than in learning	7	8	NS
Maze learning			
≤Five trials before the maze is learned	4	6	
>Five trials before the maze is learned	5	3	NS
Sentence reproduction			
All sentences of ≤20 syllables were reproduced correctly	8	8	
All sentences of >20 syllables were reproduced correctly	1	1	NS

[a] Significance was tested by Wilcoxon Matched pairs Signed Ranks test
[b] Significance is tested by Binomial test

be made in the evaluation and especially in the generalization of the findings With these restrictions in mind however there are some findings in these data which merit mention

The MD patients evidence a deficit of long term memory (retention) of non verbal material difficulties in learning of verbal material, and weakness of concentration These characteristics suggest an organic dysfunction in this patient group

The analysis studying the effect of lithium treatment on the test performance of the MD patients was inconclusive There were too few patients who were examined under both conditions and only one of 15 tests showed a significant difference The small differences which were noted suggest that the lithium treatment has, if anything, a slight negative effect on the performance of the MD patients in these tests

It is remarkable, that especially right handed patients with defect hearing in the left ear—in contrast to right handed patients with defect in the right ear—show affected functioning in verbal abstraction, long term memory of visual material, and learning of mazes There is no general intellectual deficit but it seems as if the patients with defect hearing in the left ear have a disturbance in functions presumably represented in the non dominant hemisphere The ascending pathways of the acoustic and vestibular system are almost exclusively contralateral, thus rendering it probable that a faulty processing of information in the non dominant hearing hemisphere could manifest itself from an impaired hearing and vestibular dysfunction in the left rather than in the right labyrinth

If the results obtained by methods devised in this study were to substantiate the impression of greater vulnerability of the non dominant hemisphere in MD patients it could give raise to the following speculations

Semmes (1968) has suggested that functions (simple manual capacities as well as more complex abilities) tend to be focally represented in the dominant hemisphere, but diffusely represented in the non dominant In our data the non-dominant hemisphere functions seem to have suffered greater damage Is it possible that the diffuse cerebral representations are more sensitive to the type of brain damage involved in MD disease? This interpretation would not be incompatible with an attempt to reconcile neuro psychological and psycho somatic viewpoints regarding etiological aspects of MD

About the etiology of MD we do not know if the neuro psychological disturbances are secondary to a peripheral acoustico-vestibular disorder (somato psychic) or the neuropsychological disturbances are primary to a peripheral acoustico-vestibular disorder (psycho-somatic) or perhaps a combination

In the first case are the vestibular or the acoustic dysfunction the most important? We would suggest the vestibular, because of the very wide spread connections of the vestibular system but further investigations are necessary to solve this problem

Already in 1949 McNally et al suggested that ultimately the lesion in MD may be proved to be not entirely peripheral This statement is supported from the findings of Lochen (1970) and from our results

ZUSAMMENFASSUNG

Berichtet wird uber eine neuropsychologische Studie von 19 Morbus Meniere patienten die an einem experimentellen Lithiumbehandlungsprogramm teilnahmen Die Patienten legten eine organische Dysfunktion an den Tag die nicht ausschließlich peripher war Bezuglich der atiologischen Betrachtungen der Morbus Meniere scheinen die Studienergebnisse Moglichkeiten zu enthalten die Kluft zwischen neuropsychologischen und psychosomatischen Gesichtspunkten uberbrucken zu konnen Der Lithiumeffekt wurde bewertet

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A COMPARISON OF THE FUROSEMIDE AND GLYCEROL TESTS FOR MENIERE'S DISEASE

With Special Reference to the Bilateral Lesion

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Abstract The feasibility of furosemide test for the detection of endolymphatic hydrops has previously been discussed (Authors 1973 1975) The glycerol test also has been reported as being effective for the same purpose but only in Meniere patients with fluctuating hearing loss (Klockhoff & Lindblom 1966) In 48 patients with Meniere s disease both the furosemide test (F test) and the glycerol test (G test) were performed on 51 ears including 3 cases of bilateral involvement The average value of urine volume in the F test was significantly ater than that for the G test The decrease in tinnitus 40% in the former 45% in the latter The F test elded a positive rate of 73% and the G test 45% The results were thus positive in the both tests i e F^+ G^+ were 17 (33%) F^+ G 20 (39%) F G^+ 6 (12%) and both negative F^- G only (16%) The side effects of the F test were nil but those of the G test were as follows headache (29%) nausea (4%) and increase in tinnitus (9%) The response increase of the hydropic labyrinth caused by the two kinds of systemic dehydration overlapped in part and differed in part as a result of the differing diuretic mechanisms and their respective affinities to the cochlea and the vestibulum The furosemide test may be based on the action of the vestibular response type which is caused by natriuretic dehydration accompanying the more sensitive response increase in caloric induced nystagmus while the glycerol test may be based on the action of the cochlear response type owing to osmotic diuresis manifested as hearing shift The correlation between labyrinthine hydrops and dehydration was discussed and it was concluded that these double tests were quite adequate methods for choice of treatment of not only unilateral Meniere s disease in its various stages but also in bilateral involvements

The pathology of Meniere s disease is at present described as endolymphatic hydrops in many human (Hallpike & Cairns, 19 Yamakawa, *1938*, *Schuknecht*, *1963*) a animal studies (McCabe & Wolsk, 1961, I mura, *1967*) The differentiation of M niere's disease from the other diseases w similar symptoms of different etiology shou be based on the detection of endolympha hydrops in the inner ear of the patients T feasibility of furosemide administration f detection of endolymphatic hydrops has pr viously been reported by the authors in paper on a series of 161 patients with lab rinthine vertigo including 93 typical Menie patients (1957)

Furosemide is administered intravenous as a potent natriuretic agent that produc dehydration of the inner ear, reduces the end lymphatic hydrops, and improves caloric r sponse The furosemide test yielded posit results in 80% of patients with typical M niere's disease, 6% of atypical Meniere disease (without cochlear symptom) 42 with labyrinthine syphilis 27% with sudd deafness and none with suppurative labyrintl tis As a simple diagnostic test the furosemi test is safe and effective for detection of tl hydropic state of the labyrinth

In 1966 Klockhoff & Lindblom presente the preliminary report on the glycerol te which could reveal endolymphatic hydrops i

Table I *The comparative results of the furosemide test and the glycerol test*

N=51	Furosemide test		Glycerol test		Significance level
	M (S D)	Positive rate	*M* (S D)	Positive rate	
Urine volume	753 cc (186 3)		322 cc (176 7)		T=13 12*
Decrease of tinnitus	–	18/45 (40%)		20/44 (45%)	T_a= 1 52*
Hearing shift	1 1 dB (5 38)	17/50 (34%)	4 1 dB (8 03)	23/51 (45%)	T_o= 0 02
Caloric response	13 9% (26 5)	37/51 (73%)			
Side Effect					
Headache		0		14/49 (29%)	
Nausea		0		3/49 (6%)	
Increase of tinnitus		0		4/44 (9%)	

Meniere's patients, but only those with fluctuating hearing loss Glycerol is administered orally as an osmotic agent that reduces hydrops and improves hearing

The purpose of the present investigation was to compare the effectiveness of the two dehydration tests for the confirmation of endolymphatic hydrops

SUBJECTS AND METHODS

In 48 patients with typical Meniere's disease hospitalized for the epidural shunt operation, both the furosemide test and the glycerol test were performed on 51 ears including 3 cases of bilateral involvement All the patients complained of incapacitating, frequent, long-lasting, severe attacks of vertigo, perception deafness and tinnitus and were considered to require surgical management

The furosemide test

Immediately preceding an i v injection of 20 mg furosemide, pure tone audiometry and the caloric test were performed, and subjective symptoms such as headache or tinnitus were recorded The index of hearing response was calculated as the average of the hearing thresholds recorded at 250, 500, and 1000 Hz The caloric test was performed with 50 cc of water at 30°C, and ice cold water was used in cases where no reaction was obtained with this stimulus DC nystagmography was employed to measure the maximum velocity of caloric nystagmus as the basis of evaluating labyrinthine function These tests were repeated one hour after the injection, at which time, the diuretic effect had in most cases reached its maximum The two sets of data were then compared Increase in maximum velocity of caloric nystagmus beyond the normal range (+9 4%) is referred to as positive All others are negative An average hearing improvement of greater than 5 dB was referred to as a positive co-parameter

The glycerol test

After admission, each patient underwent an immediate predosage hearing test in the morning Breakfast intake was limited 1 3 g/kg body weight of glycerol with an equal amount of physiological saline was ingested perorally This dosage was less than the original amount (1 5 g/kg) in order to reduce side effects revealed through the pilot study After ingestion, checks were made for headache, nausea, emesis, dizziness and changes of tinnitus at hourly intervals for 3 hours A final postingestion hearing test was made 3 hours after ingestion The index of hearing response was calculated as the average of hearing thresholds recorded at 250, 500 and 1000 Hz A threshold improvement of greater than 5 dB was regarded as positive, based on the original criterion No feed or liquid intake was allowed during each examining period of both tests

RESULTS

The comparative results of both tests are shown in Table I Concerning the average urine volume of each test period, the furo-

Furosemid (Cal)	
p (72 5%)	n

Glycerol (Hearing)	
p (45 1%)	n

Fig 1 Comparison of response increase *p* positive *n* negative

Table II *The results of the response types*
F the furosemide test
G the glycerol test

F(+) G(+)	17 (33%)
F(+) G(−)	20 (39%)
F(−) G(+)	6 (12%)
F(−) G(−)	8 (16%)

semide test (753 ml) was significantly greater than the glycerol test (322 ml), in spite of the shorter duration of the former (t=13 12) In 18 (40%) out of 45 patients who complained of tinnitus on the testing day, the tinnitus was diminished after administration of furosemide

In 20 (45%) out of 44 patients with tinnitus in the testing period, tinnitus was relieved after ingestion of glycerol Revealing the correlation of both tests, the statistical analysis of McNemer's t test was calculated as if the positive response (diminishing of tinnitus) in the one test also occured in the other, the negative one, and vice versa The value (t= 1 52) was significant at the level of 12%

Concerning tinnitus, there is the same tendency to diminish throughout the two different kinds of dehydration tests An improvement in hearing over 5 dB was observed in 17 (34%) out of 50 affected ears after the furosemide test and in 23 (45%) out of 51 ears after the glycerol test McNemer's test (+,+), (−,−) yielded no significant value (t_o=0 02)

The effect of furosemide on the caloric response of diseased ears was positive in 37 (73%) out of 51 ears with an average response increase of 13 9% The representative positive values were compared as follows, the F test (the furosemide test) 73% and the G test (the glycerol test) 45% (Fig 1) The results of the response types were compared as follows positive in the both tests i e F^+ G^+, were 17 (33%), F G, 20 (39%) F G 6 (12%), and both negative, F G only 8 (16%) These values of 4 cells in the two by two method are shown in Table II However McNemer's test had no significant figure (t_o=0 04) The correlation is illustrated in Fig 2 with the small correlation co efficient (r=0 03) In the F test, side effects were nil By contrast, in the G test there were remarkable side effects against the reduction of the dosage, i e headache (29%), nausea (6%), and increased tinnitus (9%)

DISCUSSION

Furosemide is structurally a sulfamyl anthranilic acid, which when administered either orally or parenterally, has very potent and rapid diuretic effects

Its diuretic action is "natriuretic", i e it depresses the reabsorption of sodium, water and chloride in both the proximal and distal tubules, including the ascending limb of the

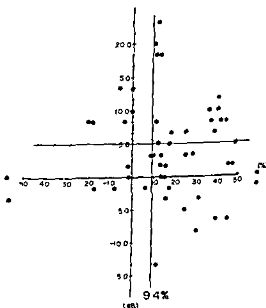


Fig 2 Illustration of the correlation between the furosemide test (*abscissa* %) and the glycerol test (*ordinate* dB) Each dot represents the values of the individual ear Criteria are shown with thin lines

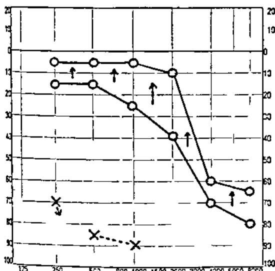


Fig 3 Hearing shift in case I in the glycerol test The burned out ear on the left did not respond positively

loop of Henle (Mushaweck & Hajdu, 1964, Stason et al , 1966)

Besides urine secretion, furosemide influences other extracellular fluids with a resulting reduction of blood pressure, a decrease of the flow of CSF, and a diminution of the pressure of CSF and chamber pressure of the eye

In accordance with these properties the increase in caloric response in hydropic labyrinth is presumed to be caused by a temporary reduction of endolymphatic hydrops by acute systemic diuresis However, as reported in our previous papers (1973, 1975), there was no significant change in hearing shift There fore despite a 34% hearing improvement in this series pertinent consideration is required to explain the increase in caloric response without concurrent hearing improvement

On the other hand, glycerol ingestion, as reported by Klockhoff & Lindblom (1968), produced hearing improvement as a result of the reduction of endolymphatic hydrops through its "osmotic diuresis, but yielded no consistent results regarding caloric response (Angelborg et al , 1971) These erratic results may be due to glycerol s alcoholic effects on the vestibular system with positional nystagmus

However the representative positive values differ as follows, 73% of the furosemide test based upon the criterion through 46 healthy

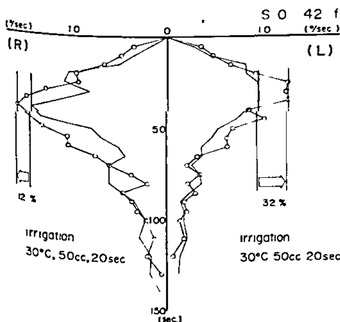


Fig 4 Caloric response of case I in the furosemide test Not only the fluctuant ear on the right but also the burned out" one on the left showed clear response increase *Abscissa* angular velocity of the slow component *ordinate* time after the end point of irrigation —— before dehydration O–O after dehydration

control ears, and 45% of the glycerol test according to the original criterion Using a 15 dB improvement criterion, Snyder reported that the glycerol test was positive in 50 out of 97 ears affected by Meniere's disease only and having a fluctuating hearing loss (1974) However, Meniere's disease is not restricted only to those with a fluctuating hearing loss In fact, among our furosemide test series, there were a few patients with the "flat loss" of the "burned out" type who responded positively in hearing tests Concerning caloric response, many of these "burned out" cases yielded positive values On comparison, the furosemide test is more sensitive than the glycerol test Concerning the results shown in Table II, "positive in both (33%) or either" totals 84% leaving only 16% of "both negative". And in the diminishing of tinnitus, a linked inclination though both tests can be obtained The increase in response of the hydropic labyrinth caused by the two kinds of systemic dehydration overlapped in part and differed in part, as a result of the differing diuretic mechanisms and their respective affinities to the vestibulum and the cochlea The furosemide test may be based on the action of the vestibular response type, which is caused by natriuretic dehydration accompanying the more sensitive response-increase in caloric induced nystagmus, while the glycerol test may be based on the action of the cochlear response type, owing to osmotic diuresis manifested as hearing shift

As mentioned above, the double checking of the hydropic state by two different dehydrations can render detection and diagnosis more sensitive and precise throughout virtually every stage of Meniere's disease with unilateral involvement Beyond these merits the double tests were of great help in the diagnosis of bilateral lesions which we now recognise as occurring in a remarkably large proportion of patients Estimation of responsibility for vertigo in bilateral involvement is rather difficult If the patient has bilateral lesions the process of the involvement of each ear can be quite complicated with different degrees of progression which usually includes one "burned out" ear

In order to explain the benefit of the double test, three cases of bilateral involvement are presented briefly

Case 1

For 3 years a 40-year-old housewife suffered vertiginous attacks with cochlear symptoms in her left ear 9 months before consultation, she noticed tinnitus and a slight hearing loss on the right, following an attack Afterwards she experienced severe and frequent attacks with progressive hearing loss in the subsequently affected right ear The earlier affected ear on the left was of the "burned out" type and did not respond in the glycerol test (Fig 3), but in the caloric response of the furosemide test the bilateral labyrinth of the patient showed clear positive results (Fig 4)

Case 2

A 42 year old housewife with the earlier affected ear on the left (for 10 years) and the later-affected one on the right (3 years) The caloric response, after the furosemide injection, increased 10% only on the right However, the hearing shift occurred not only in the ear with fluctuating hearing loss on the right (5 0 dB), but also with "flat loss' on the left (6 7 dB) The furosemide test was performed on this patient in the afternoon, and that night she noticed such a remarkable hearing improvement, with disappearance of bilateral tinnitus since the previous affection, that she was able to telephone to her husband in a distant area Next morning, she was disappointed by the return of the usual hearing of her previous condition A hearing shift of 11 3 dB in the right was obtained through the glycerol test in the afternoon Based upon the results in the double test the modified epidural shunt operation to the left ear and the steroid and diuretic treatment to the right was indicated and executed with excellent relief

Case 3

A 41 year old cab driver had begun to have mild attacks of vertigo with cochlear symptoms in his left ear for 4 years until his first consultation at our clinic in August, 1973 In spite of the depressed response, the calorization to the left ear in the furosemide test was positive (44%) with concurrent hearing shift (5 0 dB) The glycerol test was negative Consequently, later the same month, the drainage operation was carried out on his left ear followed by an attack free period without cochlear symptoms for about one year Subsequently he began to suffer momentary severe attacks with cochlear symptoms in the right ear which had been regarded as the "healthy side Since medical treatment using several drugs was resisted by the more frequent attacks over the following 9 months, an operation on the right ear was decided on in consequence The furosemide test this time showed a positive result in the subsequently affected ear on the right (27%) The previously drained labyrinth on the left now responded negatively to testing Moreover, the caloric response was recovered The glycerol test also gave no positive results

CONCLUSION

The furosemide test is certainly a more sensitive diagnostic test with fewer side effects than the glycerol test for detection of endolymphatic hydrops The response increase of the hydropic labyrinth caused by the two kinds of systemic dehydration (the former natriuretic, the latter, osmotic) overlapped in part and differed in part, depending on their respective affinities to the vestibulum and the cochlea However, the double checking can make diagnosis more firm and precise not only with unilateral involvement but also with complicated bilateral disorders Therefore by using the double test for patients with severe Meniere s disease a choice of treatment can be obtained easily and confirmedly through its specific diagnostic value

[illegible]SAMMENFASSUNG

Wir [illegible] weimal die Durchführbarkeit des Furosemid[illegible] für die Entdeckung des endolymphatische [illegible] untersucht (1973 und 1975) Es wird schon [illegible] von Klockhoff & Lindblom 1966 daß au[illegible] Glyzerintest (G Test) für dasselbe Ziel anwendb[illegible] ber er soll nur im Falle einer Menieresch[illegible] Krankheit mit einem fluktuierenden Gehörverlust [illegible] werden Auf die 51 von 48 menierischkranken Patienten die in der Universitätsklinik Kyoto behandelt wurden und von denen drei bilaterale Läsionen waren hatten wir den F und G Test durchgeführt Die durchschnittliche Menge des Urines war im F Test signifikant größer als im G Test Bei 40% der Patienten im Fall des F Tests und bei 45% im Fall des G Tests hatten wir die Verminderung des Ohrensausens festgestellt Der F Test brachte eine positive Verhältnisziffer von 73% hervor und der G Test eine von 45% Die Ergebnisse waren folgende positiv in beiden Tests i e F G waren 17 (33%) F G 20 (39%) F G 6 (12%) und schließlich beide negativ F G nur 8 (16%) Es gab keine Nebenwirkungen im F Test aber im G Test bemerkten wir folgende Nebenwirkungen Kopfschmerz (29%) Übelkeit (4%) und die Vermehrung des Ohrensausens (9%) Die Reaktionssteigerung des hydropischen Labyrinths die durch die beiden Arten der systemischen Wasserentziehung verursacht wird deckt sich teilweise und unterscheidet sich teilweise weil ihr diure [illegible]

kalorischen Nystagmus verursacht wird Andererseits kann sich der G Test auf die Verhältung des Schnecken Reaktionstyps stützen und erscheint wegen der osmotischen Diuresen als die Verschlechterung der Gehörschwelle [illegible]

Stufen [illegible] auch der zweiseitigen Läsion sein sollten

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SUR LA GAINE DES CELLULES GANGLIONNAIRES DE SCARPA CHEZ LE BABOUIN *PAPIO PAPIO*

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Abstract La gaine des cellules bipolaires du ganglion vestibulaire de Scarpa chez Papio papio est constituee par plusieurs cellules de Schwann qui forment de la myeline lache et par endroits un petit nombre (jusqu a 5) de couches de myeline compacte La plupart des couches cytoplasmiques de Schwann s arretent au niveau de l'émergence des neurites et forment en ce point un demi noeud de Ranvier incomplet La constitution de la gaine du perikaryon du protoneurone vestibulaire est inter médiaire chez Papio entre les aspects decrits antérieure ment chez le Rat et chez l Homme

Les cellules ganglionnaires de la huitieme paire cranienne sont chez la plupart des ver tebres etudies jusqu'a présent (Munzer, 1931, Rosenbluth, 1962) entourées par une gaine de myeline ou de myeline lâche L'Homme con stitue une exception a cette regle puisque nous avons pu montrer (Perre et al 1975) que le corps cellulaire du ganglion de Scarpa humain est entoure de cellules satellites qui ne forment que tres rarement un ou deux replis sans myeline vraie Dans le but de rechercher des etats intermediaires entre celui observé chez l Homme et celui connu chez les autres vertébres nous avons entrepris d etudier le ganglion vestibulaire chez les primates et tout d abord chez *Papio papio*

MATERIEL ET METHODES

Deux animaux adultes ont ete etudies apres fixation (dans un cas par immersion (parafor maldehyde) dans l autre par perfusion selon Karnovsky), postfixation au tetraoxyde d osmium, inclusion en Araldite Les coupes fines ont ete examinees au microscope elec tronique Siemens apres avoir été contrastees a l acetate d uranyle et au citrate de plomb Pour les etudes quantitatives, des coupes provenant du deuxieme prelevement ont été recueillies sur membrane de formvar et étu diees sur porte objet à trou unique

RESULTATS

La gaine des cellules ganglionnaires depend de cellules de Schwann Il est suffisamment fre quent que deux noyaux schwanniens soient interesses par une même coupe fine pour que l'on puisse conclure que la plupart des corps cellulaires neuronaux sont entoures par plusieurs cellules de Schwann

Certaines cellules sont entourees d une gaine de myeline lâche On entend sous ce terme (loose myeline, Rosenbluth & Palay, 1961) l'empilement de cytoplasmes schwan niens en lames d epaisseur
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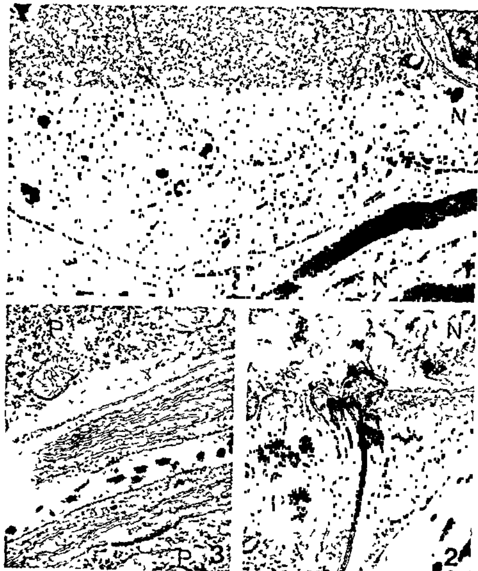


Fig 1 Partie d un perikaryon myelinise, avec l'émergence d un des deux neurites Au voisinage partie d un neurite myélinisé ×6600

P Perikaryon neuroganglionnaire *S* Cellule de Schwann *N* Neurite

Fig 2 Detail de la precedente La gaine du périkaryon comporte de la myéline lâche, trois feuillets de myéline compacte et un feuillet cytoplasmique schwannien Seul ce dernier se prolonge sur le neurite émergent ×34000

Fig 3 Myéline semicompacte recouvrant deux périkaryons Entre les deux, basales et fibres collagènes ×29000

nant un renversement de direction de la languette On observe souvent que la languette schwannienne s'amincit et que ses deux membranes plasmatiques fusionnent pour former une ligne dense qui peut rester isolee ou contribuer à former une couche de myéline soit semi-compacte, c'est a dire a bande claire large et irrégulière et sans ligne intermediaire, soit compacte avec des caracteres identiques a celle des gaines des neurites, mais toujours formée d'un petit nombre de feuillets (5 au plus) Ce n'est qu'autour d'un petit nombre de cellules (7, sur 75 dont la gaine entière pouvait être observée dans des conditions optimales) que la gaine comportait sur toute son étendue de la myéline compacte, toujours doublée de myéline lâche.

A la surface d'un petit nombre de cellules ganglionnaires on observe des prolongements cytoplasmiques groupés, de section arrondie,







Fig 4 Gaines d'un neurite (63 feuillets) et d un périkaryon (5 feuillets) ×35 000
Fig 5 Passage de la myéline lache a la myeline compacte ou semicompacte La ligne dense correspond à l'aplatissement d'une languette cytoplasmique ×55 000
Fig 6 Enroulements de myeline semicompacte ×29 000

contenant ribosomes et vesicules et parfois réunis au cytoplasme neuronal par un contact specialise symétrique

Au voisinage de l'emergence des deux neurites, qui prennent naissance sur ces cellules bipolaires, on observe une formation particuliere analogue à un noeud de Ranvier partiel · les lames schwanniennes et les

feuillets myeliniques se replient vers le grand axe de la cellule, les lignes denses font place a du cytoplasme, et les unes et les autres se terminent en alignement approximativement parallele au grand axe Cette disposition a pour effet un certain etranglement de la cellule marquant le passage du perikaryon au neurite Au dela et jusqu'au noeud de Ranvier qui marque le debut de la gaine myelinique normale, le prolongement neuronal n'est entoure que par deux a trois feuillets de myeline lache, qui même ne persistent pas jusqu'au noeud lui même Celui ci se trouve ainsi forme pour une moitie par un simple repli schwannien, l autre moitie etant formee normalement par les replis successifs issus de la gaine de la fibre Le dernier de ces replis s'etend plus loin de la gaine qu'il n'est habituel donnant au premier abord l'impression d'un espace nodal exageré

DISCUSSION

Nous avons comparé nos resultats a ceux des etudes ultrastructurales du ganglion de Scarpa existant dans la litterature, c'est a dire essen " a ceux de Rosenbluth (1962) chez le de Ballantyne & Engstrom (1969) chez le cobaye et accessoirement Saimiri sciurus, et de Perre et al (1975) chez l Homme Nous avons juge utile de reprendre des points de detail directement sur deux rats perfuses et inclus selon les memes techniques, car Rosenbluth utilise une fixation differente (osmique directe) et ne distingue pas dans sa description ganglions vestibulaire et cochleaire, la plupart des illustrations etant empruntees a ce dernier ganglion Dans l ensemble la situation que nous trouvons chez *Papio papio* est intermediaire entre celle de l Homme et celle du Rat chez l Homme sur 30 cellules observees completement aucune n avait de myeline vraie, et 2 ou 3, selon les criteres retenus de la myeline lache Chez le Rat sur 39 cellules 4 sont entourees de myeline lache et 35 de myeline compacte ou semi compacte Le nombre de feuillets de myeline qu ind elle existe, est aussi plus reduit chez Papio papio que chez le Rat ou on compte de 8 a 14 feuillets

Sur le corps cellulaire, le comportement de la myeline et de la myeline lâche est identique a celui décrit longuement par Rosenbluth nous n'y reviendrons pas, non plus que sur les consequences que ces observations comportent pour la theorie de la formation de la myeline Par contre les structures que nous avons observées au niveau de l emergence des neurites — sans que nous puissions avec certitude distinguer neurite central (axone) et peripherique (dendrite) — ne sont observées ni chez l Homme, ou la gaine de Schwann se continue simplement sur le neurite, ni chez le Rat, ou la gaine de myeline ne presente à ce niveau pas plus d irregularité qu ailleurs Le resultat en est que, chez Papio, sur une longueur de 2 a 3 microns qui represente la distance entre le perikaryon et le premier noeud de Ranvier, la cellule nerveuse n est separee de la basale que par un petit nombre de lamelles de cytoplasme schwannien le plus souvent une seule qui continue la plus periphérique de celles qui entourent le perikaryon Cette disposition etagee du noeud de Ranvier est repetee a un moindre degre de l autre coté de ce noeud asymetrique, elle nous a paru très particulière elle amorce en quelque sorte sur le segment initial du neurite la situation qui sera uniformément celle du perikaryon vestibulaire de l Homme

Au total, nos observations confirment indirectement, en amorçant une serie phylogenique, les conclusions de Perre et coll concernant l Homme, chez qui les conditions de prelevement pouvaient suggerer une origine pathologique des images

Elles appellent une etude systématique de la gaine des cellules ganglionnaires de Scarpa dans la série des primates, car la variance interspecifique de cet organite pourrait constituer un nouveau fil conducteur dans l etude des rapports phylogeniques au sein de cet ordre

(Technique photographique Monsieur Le Cren I N S E R M)

SUMMARY

The sheath of the bipolar perikarya of the vestibular ganglion (Scarpa) in *Papio papio* is made up of several Schwann cells which concur to form loose myelin and at most five layers of compact myelin Most of the Schwann cytoplasmic layers stop at the emergence of both neurites forming at that point an incomplete Ranvier half node The constitution of the vestibular perikaryal sheath in Papio is intermediate between those previously described in the Rat and in the Human

ZUSAMMENFASSUNG

Die Scheide der bipolaren Ganglienzellen des Ganglion vestibulare (Scarpa) besteht aus mehreren Schwann schen Zellen, diese Zellen bilden lockeres Myelin und in Stellen einige (bis 5) Schichten von dichtem Myelin Die meisten Schwannschen zytoplasmischen Schichten sind an der Stelle des Heraustauchens beider Neuriten unter brochen Sie bilden dort einen unvollstandigen halben Ranvier Ring Die Zusammensetzung der Perikaryon scheide des vestibularen Protoneurons liegt bei Papio zwischen denjenigen, welche vorher bei Ratte und Mensch beschrieben wurden

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SCANNING ELECTRON MICROSCOPIC STUDY ON THE DISTRIBUTION OF EPITHELIAL CELLS IN THE EUSTACHIAN TUBE

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(Received January 9 1976)

Abstract Canine Eustachian tube epithelium was examined by means of the scanning electron microscope The part of the tube at the bone–cartilage junction was found to be the most active It is here that goblet cells and large numbers of ciliated cells were found Cilia were dense and covered by a mucus blanket Near the tympanic end of the Eustachian tube goblet cells were more numerous and ciliated cells less so Near the pharyngeal end goblet cells were numerous while cilia were scanty and not uniform in length Our findings support the concept that middle ear clearance is carried out by an active mucociliary mechanism as in other parts of the upper respira y system

Many reports on the gross anatomy, histology and physiology of the Eustachian tube are to be found in the literature Most reports dealing with the Eustachian tube are concerned primarily with its function and whether it is normally opened or closed Recently electron microscopic studies have been reported (Shimada & Lim, 1972, Hentzer, 1970 Lim et al, 1967)

The Eustachian tube epithelium is known to consist of pseudostratified ciliated and non ciliated columnar cells goblet cells and underlying basal cells resting on a thin basement membrane However the frequency and distribution of the ciliated and goblet cells along the Eustachian tube and the length and arrangement of cilia have not been fully investigated

In this study the canine Eustachian tube epithelium was studied at different levels using the scanning electron microscope The study was intended to elucidate the mechanisms of middle ear clearance as suggested by Sadé et al (1970) to be carried out by an active mucociliary system It also provides a means of comparison for future studies on experimental pathological conditions in Eustachian tubes in dogs

METHODS AND MATERIAL

Temporal bones of dogs with clinically healthy ears were dissected out through the preservation of Eustachian tube Immediate fixation was done in 2% glutaraldehyde buffered at pH 7 4 with Millonig's solution for 24 hours Post fixation was made in 1% osmium tetroxide solution for 2 hours Tissues were trimmed in 70% alcohol under an operating microscope and dehydrated in ascending grades of alcohol then placed in a few changes of isoamyl acetate for 30 minutes For observation of surface structures the critical point drying method using carbon dioxide was applied For observation of intracellular structures the specimens were frozen with liquid nitrogen Frozen tissues were then broken by a knife and the Ion etching method applied After



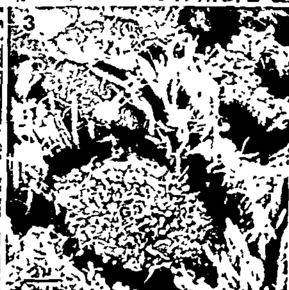


Fig 1 Eustachian tube epithelium at the junction of bony and cartilaginous parts showing dense and uniform cilia

Fig 2 Hair like filaments branching out from sides and top of cilia

Fig 3 Goblet cell (*G*) with granulated surface and short microvilli

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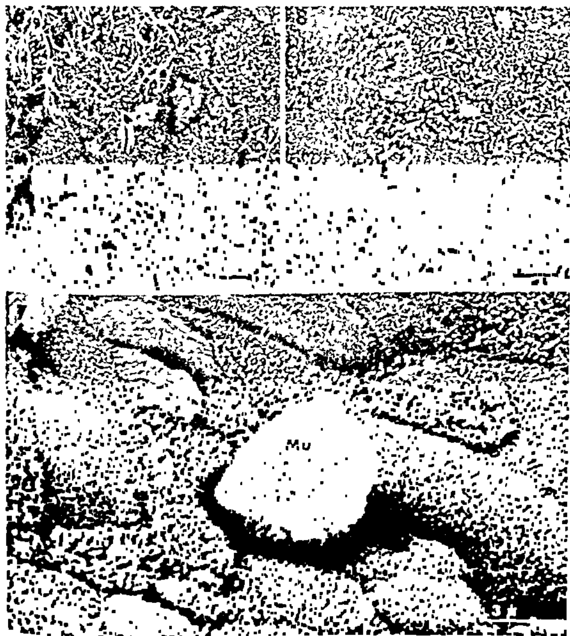


Fig 6 Eustachian tube epithelium near the pharyngeal end showing cells covered with microvilli and few irregular cilia Goblet cells are numerous

Fig 7 Ductal opening of gland with mucus (Mu) issuing

Fig 8 Flattened epithelial cells (St) in between columnar cells

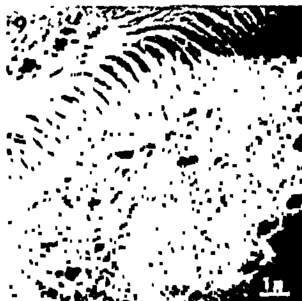
Fig 9 Cut surface of ciliated columnar cells showing uniform cilia covered by mucus blanket The nucleus (N) is rounded

Fig 10 Nucleus (N) of ciliated columnar cell showing fine pores Mitochondria (Mi) are present around the nucleus

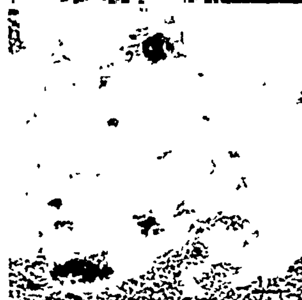
Fig 11 Mature goblet cell with large number of secretory granules (Sg)

Fig 12 Basal cells (B) wedged between bases of ciliated (C) and goblet cells (G) N nucleolus

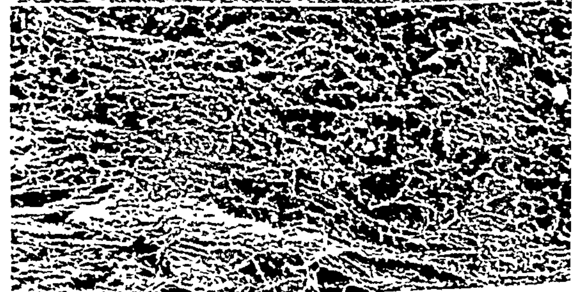
Fig 13 Collagenous fibres in the subepithelial layer


9
1μ


N




12
C
N



II Cut Surface of Eustachian Tube Epithelium

A Ciliated columnar cell (Fig 9)

The free surface was lined with a uniformly dense layer of cilia Each cilium measured about 3 μm in length Over the ciliate surface a thick mucous layer was seen The cytoplasm appeared vacuolated with condensation of mitochondria and granules in both the supra and infra nuclear portions The nucleus was rounded, with fine pores throughout its walls (Fig 10) It was usually located in the middle third of the cell The cell boundaries were smooth except at the basal part where finger like processes were found

B Goblet cell (Fig 11)

The matured goblet cell had the classically described goblet shape with expanded supra nuclear portion and a tapered basal end The cytoplasm contained a large number of spherical or oval secretory granules Some of these granules covered the free surface of the cell Some goblet cells appeared in an immature or inactive form The cytoplasm of these cells contained no secretory granules and the cells were shrunken and slender

C Basal cell (Fig 12)

Small triangular or quadrangular cells formed two or three layers These were wedged between the bases of ciliated and goblet cells resting on the basement membrane The cytoplasm was dense, small in amount

In the subepithelial layer, collagenous fibres were scattered or formed small bundles running in all directions but mostly in the longitudinal direction of the Eustachian tube (Fig 13)

DISCUSSION

It is well known that the muco ciliary mechanism of the respiratory epithelium is the major defense system for clearing foreign particles from the surface of the air exposed cavities such as the nose and trachea This mechanism was found to be not only mechanical in nature but is also a complex immunochemical defense system (Butler et al , 1967) The middle ear was also observed to possess a similar muco ciliary transportation system (Sade 1967) Furthermore it was confirmed that there are certain tracts in the middle ear cavity which serve as active drainage systems towards the Eustachian tube (Allam 1969, Shimada & Lim 1972)

In this study our findings regarding the population and distribution of ciliated cells in the mucous membrane of the normal Eustachian tube of dogs have thrown some light on middle ear and Eustachian tube clearance The large population of active ciliated cells was found in the narrow part of the Eustachian tube which lies at the junction of bone and cartilage This part seems to play the major role in this clearance mechanism The active and uniform ciliary action in this segment would drive the mucus blanket from the middle ear cavity and tympanic part of Eustachian tube towards the nasopharynx, where it will join the nasal mucus flow This clearance action of the Eustachian tube would also prevent the ascent of micro-organisms present in the nose and nasopharynx to the middle ear cavity The pattern of ciliated cells and goblet cell distribution and their mutual importance prompted us to view them as one functional unit

ZUSAMMENFASSUNG

Es wird das Epithel der Eustachischen Röhre des Hundes mit einem Rasterelektronenmikroskop geprüft Untersuchungen der Rohre ergeben daß das Epithel uber der Verbindung von Knochen und Knorpel von großter Aktivitat ist An d eser Stelle findet man Becherzellen und zahlreiche Flimmerzellen Die Zilien sind dicht und mit "mucus Blanket bedeckt In Nahe des tympanischen Endes der Eustachischen Röhre gibt es mehr Becherzellen und weniger Flimmerzellen In Nähe des Rachenendes gibt es zahlreiche Becherzellen wohingegen die Zil en sparlich und nicht gleichlang waren Unsere Ergebnisse unterstutzen die Hypothese daß d e Mittelohrreinigung ebenso wie an anderen Teilen der oberen Luftwege durch einen aktiven muko-ziliaren Mechanismus durchgeführt wird

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ULTRASTRUCTURAL CHANGES OF THE NERVE ELEMENTS FOLLOWING DISRUPTION OF THE ORGAN OF CORTI

I *Nerve Elements in the Organ of Corti*

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Abstract 3–137 days after disruption of the guinea pig organ of Corti by perilymphatic perfusion with 20% streptomycin (SM) ultrastructural changes of the nerve fibers in the organ were observed Most of nerve fibers began to degenerate after a latent period of 4 days On the other hand a number of fibers survived reactively enlarged and later developed into myelinated and unmyelinated fibers by becoming enclosed in Schwann cells which entered the organ of Corti through the habenula perforata Regeneration and sprouting of the surviving nerve fibers also occurred The fibers became mature but atrophied after 60 days and then gradually disappeared The regenerating fibers were mainly of the myelinated and unmyelinated efferent type Retrograde degeneration occurred in both afferent and efferent fibers In the less damaged organ of Corti perfused with 2% SM or Ringer's solution Schwann cell invasion was not found

There are many factors capable of causing damage to the cochlea The grade of morphological damage found in the cohlea ranges from minimal cytoplasmic changes (Duvall et al , 1969) to bony obliteration of the cochlear duct (Bernstein & Silverstein, 1966) The fate of the cochlear nerve fibers in the damaged organ of Corti is of great interest because of a new treatment for sensory deafness which has recently been developed by Diourno et al (1957) and Michelson (1971) The principle of this treatment is based on electrical stimulation of the surviving cochlear nerve fibers using an electrode wire implanted in the scala tympani through the round window Histological changes in the damaged organ of Corti, especially those involving the cochlear nerve fibers, are obscure Except for those related to acoustic trauma, few electron microscopic studies have been reported regarding these nerve fibers in pathological situations

Retrograde degeneration is accepted as one of the characteristics of the cochlear nerve if the organ of Corti is destroyed the degeneration of the cochlear nerve fibers ascends centralward (Wittmaack, 1932, Schuknecht, 1953, Kellerhals et al , 1967, Spoendlin, 1971) In the present electron microscopic study, a perilymphatic perfusion of a mixture of dihydrostreptomycin and streptomycin sulfate solution (combined streptomycin, abbreviated as SM) was used to disrupt the organ of Corti of the guinea pig We then examined the ultrastructural changes in the nerve elements in the cochlea

Two interesting findings were observed which have not been reported previously In the normal organ of Corti, all nerve fibers are naked axons devoid of Schwann sheath They become myelinated or unmyelinated nerve fibers by being enclosed in the sheath of

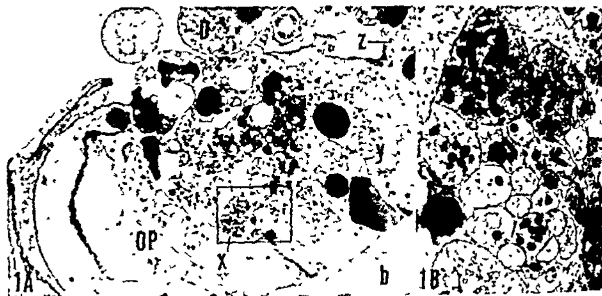


Fig. 1. 3 days after perfusion 20% SM. 1st turn. (A) severe disruption of the outer hair cell area. Three outer spiral bundles (x, y, z) still remain. *OP*: outer pillar, *D*: Deiters cell, *b*: basilar membrane. ×3000. (B) Higher magnification of a bundle *x* in (A). The fibers appear almost normal. Two of them on the left are filled with vesicles and mitochondria. ×14000.

Schwann cells just proximal to the habenula perforata. Following disruption of the organ of Corti, Schwann cells enter the damaged organ of Corti along the surviving nerve axons. Thus myelinated and unmyelinated nerve fibers develop in the organ of Corti. Temporary regeneration or sprouting of the surviving nerve fibers occurs in the disrupted organ of Corti.

Morphological changes in other cell types in the organ of Corti and in the spiral ganglion cells and nerve fibers in the area proximal to the organ of Corti of these animals will be reported separately.

MATERIALS AND METHOD

Young albino guinea pigs (200–300 g) with normal pinna reflexes were used. They were anesthetized with an intraperitoneal injection of nembutal and a local injection of carbocain into the periauricular area. The bulla was opened by a retroauricular approach. Under an operating microscope a hole was drilled into the rim of the oval window. SM solution was used at two different concentrations, 2% and 20%. Each 1 g SM contained 0.5 g dihydrostreptomycin and 0.5 g streptomycin sulfate. Diluted in Ringer's solution, it was gently perfused into the scala tympani through the round window membrane until the solution flowed out from the drilled hole adjacent to the oval window. After the perfusion, the tympanic cavity was cleaned and the skin incision closed.

As a first group, 28 cochleae of 18 animals were perfused with 20% SM solution bi- or unilaterally. The postoperative survival time of the animals from this group was 3 to 137 days. As a second group, 8 cochleae in 4 animals were perfused with 2% SM solution by the same procedure as described above. The animals were sacrificed 31 to 76 days postoperatively. In addition, 16 cochleae were perfused with Ringer's solution only. The survival time of this group was 7 to 137 days.

The animals of all groups were sacrificed by intraortic perfusion using 2.5% cacodylate buffered glutaraldehyde solution at pH 7.4. Some were anesthetized with nembutal and the cochleae were perfused through the round window with the fixative. The cochleae were

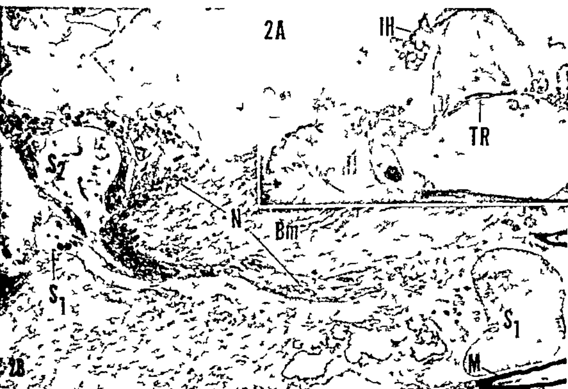


Fig 2 4 days after perfus on 20% SM 1st turn (A) In the organ of Corti outer ha r cells are m ss ng Inner hair cell (*IH*) s degenerat ng The nerve bundles have kept the r normal pos t on *IP* nner p llar *TR* tunnel rad al f bers ×900 (B) H gher magn f cat on of the habenula perforata (arrow n A) Schwann cells (S_1 S_2) wh ch normally do not pass through the habenula perforata enter the organ of Corti with p riform swell ng Note one of the cells (S_2) encloses nerve f bers *Bm* bas lar mem brane *N* nerve f bers *M* myel nated nerve f bers ×3 600

emoved and postfixed in 1% phosphate buffered osmium tetroxide for an hour The specimens were dehydrated in acetone and embedded in Epon 812 Sectioning was done with an LKB Ultrotome The sections were examined using the Hitachi HS 7 or HU 12 electron m croscope

FINDINGS

Gross histologic changes of the organ of Corti after perilymphatic perfusion with 20% SM showed that the organ of Corti was severely disrupted at an early stage and later was collapsed on the basilar membrane into a hump-l ke mass The speed and grade of the changes varied to some extent in the indi vidual cochlea The limbus and collapsed organ of Corti mass were covered by a single cell layer continuous with Reissner s mem brane The mass appeared to conta n only the tectorial membrane and fibrocytes

Ultrastructurally at 3 and 4 days after perfu sion all supporting cells in the organ of Corti were severely disrupted The outer hair cells could not be found The inner hair cells were degenerating (Figs 1A 2A) Among these cells bundles of nerve fibers remained but the nerve endings had disappeared Most of the nerve f bers d d not show distinct degenera tion although they were slightly reduced in number (Figs 1 2) At 4 days Schwann cells in the habenula perforata were observed to en large and elongate through the habenula along the nerve f bers into the organ of Corti Their distal processes showed a pyriform enlarge ment (Fig 2)

At 5 days due to the progress of cellular


3


A
M
U
Q
P
S
H
5

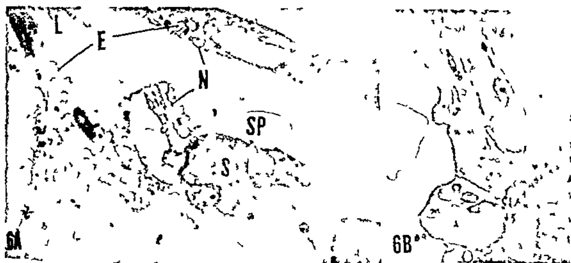


Fig 6 (A) The same section as Fig 5 showing the vestibular lip (*L*) of the spiral limbus covered by a single cell layer (*E*) continuous with Reissner s membrane Extension of the nerve fibers (*N*) on the surface of the limbus and into the empty spaces (*SP*) produced by disappearance of the interdental cells *S* Schwann cell in phagocytosis ×4 500 (B) Higher magnification of the nerve fibers (A) in (A) Note reappearance of neurotubules in axons and lack of basement membrane ×15 000

disruption the organ of Corti began to collapse The number of degenerating nerve fibers increased markedly They were either shrunken into a dense mass or swollen into a homogeneous substance containing myelin figures dense debris enlarged endoplasmic reticulum and swollen mitochondria (Fig 3) Among the degenerating fibers there were still many fibers with normal appearance

From 7 days enclosure of axons by Schwann cells began from the nerve fibers coursing along the basilar membrane Most of the remaining nerve fibers became enormously thick in diameter and showed scattered dense bodies numerous neurofilaments disappearance of neurotubules and collecting of mitochondria in their axoplasms A cone like enlargement of the axons was sometimes encountered (Fig 4) This enlargement of the axons was most prominent from 9 to 17 days

At 10 days macrophages appeared in the organ of Corti The degenerated nerve fibers were often digested by macrophages (Fig 5) or Schwann cells (Fig 6) By 12 days all the nerve fibers in the organ of Corti were enclosed by Schwann cell processes into individual axons or bundles (Figs 4 5 6 7)

At 14 days, Schwann cells began to form myelin lamellae around their axons (Fig 5) The number of the myelin lamellae increased with survival time i e 6 lamellae in the 14-day specimen 9 in the 20-day and 21 in the 35 day Axons of the unmyelinated fibers frequently showed varicose enlargements and clusters of agranulated and granulated vesicles in their axoplasms (Fig 9 A)

Twelve to 20 days after perfusion the nerve

Fig 3 5 days after perfusion 20% SM 2nd turn Degenerating fibers (*l*) in the inner spiral bundle show enlarged neurotubules and endoplasmic reticulum myelin figures dense bodies and swelling of mitochondria ×12 000

Fig 4 12 days after perfusion 20% SM 1st turn Nerve fibers (A) surviving in the organ of Corti are enclosed by Schwann cells (*S*) Pyriform enlargement of axons (A A_1) appear to be a growth cone of the regenerating axonal sprout The [illegible]

[illegible] ung nerve fibers differentiate into myelinated (*M*) with 6 myelin lamellae and unmyelinated (*u*) fibers Note increase in thickness of axons (*A*) *H* habenula perforata *S* Schwann cell *P* macrophage *E* a single cell layer covering the collapsed organ of Corti *D* degenerating cell ×6 900

Fig 7 17 days after perfusion 20% SM 2nd turn Schwann cell processes (*S*) are *enclosing the remaining* nerve fibers Smaller fibers grouped in a *bundle of naked* axons (*l*) *b* basement membrane *T tectorial* membrane *E* a single cell layer ×15 000

Fig 8 20 days after perfusion 20% SM *1st turn* Myelinated (*M*) with 9 myelin lamellae and unmyelinated (*U*) fibers extend along the lateral surface of the vestibular lip (*L*) of the spiral limbus *b* basement membrane ×11 400

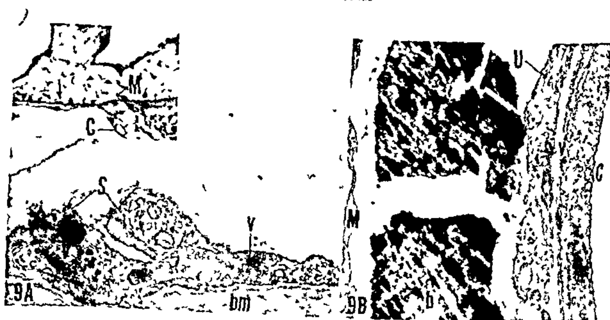


Fig 9 35 days after perfusion 20% SM 2nd turn (*A*) vesicle filled and varicose axons of *mature* unmyelinated nerve fibers on the basilar *membrane* (*bm*) indicated by an arrow in the inset *v* vesicles *S Schwann* cell Note a myelinated fiber (*M*) within the habenula perforata in the inset ×18 000 (B) An unmyelinated (sympathetic) nerve fiber (*U*) along the blood vessel (*C* in the inset) Note reappearance of neurotubules and vesicles *M* mesothelial cell *b* bone ×13 800

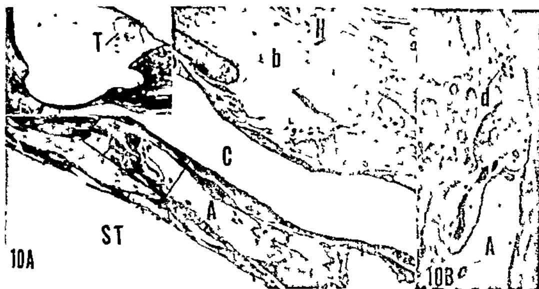


Fig 10 10 days after perfusion 20% SM 1st turn (A) An unmyelinated (sympathetic) nerve fiber (A) coursing along the blood vessel (C) on the scala tympani (ST) surface of the basilar membrane (b) Its axons are abnormally thick H habenula perforata ×3300 (B) Higher magnification of axons of the sympathetic fiber in (A) Note increase of neurofilaments and debris (d) ×8600

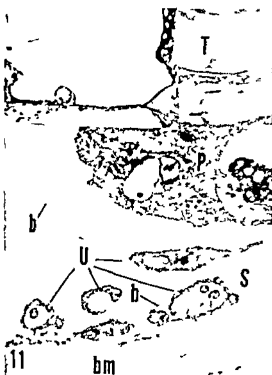


Fig 11 64 days after perfusion 20% SM 2nd turn Atrophy of the unmyelinated nerve fibers (U) on the basilar membrane (bm) results in space formation between the basement membrane (b) and Schwann cell (S) Number of axons in a single Schwann cell decreased Vesicles are few T tectorial membrane P macrophage ×13 ?00

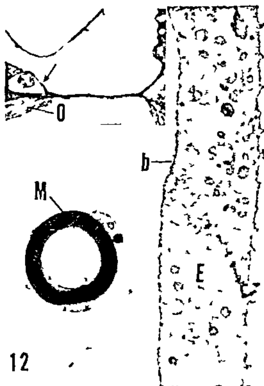


Fig 12 137 days after perfusion 20% SM 2nd turn A matured myelinated nerve fiber (M) indicated by an arrow in the inset Its myelin lamellae is thick Note the almost empty osseous spiral lamina (O) E a single cell layer b basement membrane ×12000



Fig 13 76 days after perfusion 2% SM as control 1st turn Only an outer hair cell remains (see the inset) The inner spiral bundle (*I*) shows empty spaces due to disappearance of fibers No invasion of Schwann cell *t* tunnel spiral bundle *IP* inner pillar *IH* inner hair cell ×6400

fibers remaining in the habenula perforata and organ of Corti were gradually reduced in number In the 17 day specimen severely degenerated nerve fibers were no longer observed

The interdental cells of the spiral limbus showed progressive degeneration at the initial . By 10 days after perfusion most of them d disappeared and flask shaped empty aces remained at the upper border of the spiral limbus Both within these spaces and on the surface of the limbus where no nerve fiber normally exists nerve fibers were observed (Figs 6 8) The basement membrane which surrounds Schwann cells and axons were missing at the early stage but became complete in the 20 day animal (Fig 8)

In the 35 and 64 day specimens the thickness of Schwann cells of unmyelinated nerve fibers and the number of axons enclosed within a single Schwann cell decreased (Figs 9A 11) The size of the axons of the nerve fibers was also reduced Until 35 days post perfusion, clusters of vesicles were present in the axons of unmyelinated fibers (Fig 9A) Sixty four days after perfusion however vesicles were only occasionally found in the axons (Fig 11) Additionally the surviving nerve fibers had shrunk prominently so that the space between the basement membrane and Schwann cell was enlarged (Fig 11) In the 137 day specimen only a few nerve fibers were found in the organ of Corti (Fig 12)

The sympathetic nerve fibers along the blood vessels under the basilar membrane appeared normal at the early stage following perfusion Then they presented an increase in size of the axons and a disappearance of neurotubules (Fig 10) In the 35-day specimen however, the fibers again exhibited a normal appearance (Fig 9B)

The less damaged organ of Corti perfused with 2% SM or Ringer s solution retained its normal contour without collapse The outer or inner hair cells were often missing They were replaced by Deiters cells and inner supporting cells respectively Nerve fibers grouped in bundles in the organ of Corti showed a marked decrease in number (Fig 13) However, invasion of Schwann cells in the organ was never observed Accordingly the fibers remained in naked axons Nerve fibers coursing in unusual areas were not found

DISCUSSION

Specific affinity and toxicity of streptomycin and dihydrostreptomycin to the inner ear especially to the sensory cells has been estab

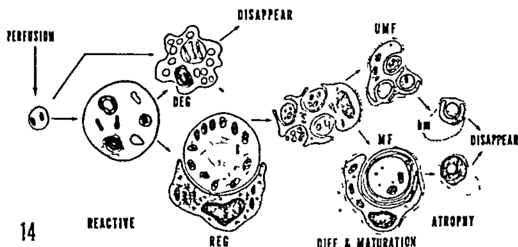


Fig 14 Stages of ultrastructural changes of the nerve fibers in the damaged organ of Corti After a latent period of 4 days reactive stage begins Most nerve fibers degenerate (*DEG*) and are surrounded by Schwann cell (S) The fibers differentiate (*DIFF*) into myelinated (*MF*) or unmyelinated (*UMF*) nerve fibers with basement membrane and then maturation occurs in a similar manner to embryonic development of nerve fibers Finally the nerve fibers atrophy and disappear

lished by electron microscopic and autoradiographic studies (Duvall & Wersall, 1964, Balogh et al, 1970, Ilberg et al, 1971, Portmann et al, 1974) In our present study, perilymphatic perfusion with 20% SM solution caused severe disruption of the organ of Corti The disruption seemed to be induced mainly by the ototoxicity of dihydrostreptomycin and streptomycin sulfate The local concentration of SM in the perilymph by this perfusion method is much higher than in the case of general administration

Ultrastructural changes of the nerve fibers in the organ of Corti damaged by 20% SM perfusion are summarized as follows After a latent period of 4 days, some of the nerve fibers degenerate and disappear, while other fibers survive The surviving fibers temporarily enlarge and are enclosed by Schwann cells which entered from the habenula perforata Then they differentiate into myelinated or unmyelinated nerve fibers Decrease in number and size of the fibers steadily progresses until these newly developed fibers disappear

Except for the latent period in the early stage and atrophy in the last stage, these processes principally correspond to degenerative and regenerative processes in the proximal stump of the transsected sciatic nerve (Wechsler & Hager, 1962) and in the transsected dorsal column of the rat (Lampert, 1967) in the points of an appearance of macrophage, reactive enlargement of axons, encapsulation of regenerating axons by Schwann cells and development of nerve fibers into myelinated and unmyelinated fibers

In addition, sprouting of the regenerating nerve fibers was also observed Fourteen days after perfusion nerve fibers were found on the upper surface and within the empty spaces of the spiral limbus where normally there is no nerve fiber Since these spaces were filled with degenerating interdental cells shortly after perfusion, the nerve fibers could not enter the spaces We consider these fibers to show the

nerve fibers which survived the damage, regenerated and actively elongated into the spaces after disappearance of the interdental cells Also, a cone like enlargement of the axonal tip was observed (Fig 4) The enlargement seems to correspond to a growth cone of the regenerating sprout described by Lampert (1967)

From 4 days after perfusion, Schwann cells entered the organ of Corti through the habenula perforata and enclosed the nerve fibers remaining in the damaged organ of Corti Later, the fibers differentiated into myelinated or unmyelinated nerve fibers in the same manner as in the maturation process of general peripheral nerve fibers during embryonic development (Wechsler & Hager, 1962, Nakai & Hilding, 1968, Allt, 1969) The process is schematically illustrated in Fig 14

On the other hand, although a number of nerve fibers still survive, invasion of Schwann cells was not observed in the slightly damaged organ of Corti which had been perfused with 2% SM or Ringer's solution only Therefore, Schwann cell invasion would occur only when the grade of injury to the organ of Corti or abenula perforata is optimal for the invasion

Because the Schwann cells enter the organ of Corti from the osseous spiral lamina along the surviving nerve fibers myelinated and unmyelinated fibers would be derived respectively from the same kinds of nerve fibers as in the central areas It is of great interest to identify the origin of the surviving myelinated and unmyelinated fibers The afferent cochlear nerve is composed of myelinated fibers among which a small number of unmyelinated fibers of unknown character are scattered Generally, they are believed to degenerate after disruption of the organ of Corti due to retrograde degeneration It is however difficult to say that the cochlear nerve fibers do not survive at all, because a few spiral ganglion cells and a small number of nerve fibers are still found in Rosenthal s canal modiolus and internal acoustic meatus at 137 days after perfusion

The efferent nerve fibers to the organ of Corti (olivo cochlear bundle) consist of myelinated and unmyelinated fibers in the area central to the organ of Corti (Terayama et al, 1969, 1971, Maw, 1973) After perfusion with 20% SM solution, degeneration of efferent fibers in the Rosenthal s canal was also found but was less extensive and slower than that of the afferent nerve fibers—as will be reported elsewhere Most of the myelinated fibers in the disrupted organ of Corti are probably myelinated efferent fibers

The unmyelinated efferent fibers in the area central to the organ of Corti (Terayama et al 1969, 1971, Maw, 1973, 1974) as well as the efferent preterminal fibers inside the organ of Corti (Spoendlin, 1966, Smith, 1967) display varicose enlargements of axons and accumulation of agranulated and granulated vesicles in the axoplasms In the present study, the axons of unmyelinated fibers surviving in the collapsed organ of Corti frequently showed varicosities and accumulation of the vesicles This seems to be indirect evidence that the surviving unmyelinated fibers were derived from the unmyelinated efferents

Duvall et al (1969) described that mechanical disruption of the organ of Corti resulted in degeneration of both afferent and efferent nerve fibers In this study, since only a few fibers ultimately remained in the organ of Corti, one should consider that retrograde degeneration occurs not only in afferent but also in efferent fibers

Invasion of Schwann cells and regeneration of nerve fibers appeared to be very active until a month after perfusion Then the once matured nerve fibers gradually decreased in size and number until they reached a point at a further advanced stage where they would not be able to function It was seen on the other hand, that the less damaged organ of Corti perfused with 2% SM or Ringer s solution contained a considerable number of remaining nerve fibers after perfusion, possibly enough to respond to the artificial stimulation

Terayama et al (1966, 1969 and 1970) and

recently Densert (1974) reported the presence of perivascular sympathetic nerve fibers (adrenergic fibers) on the blood vessels of the scala tympani surface of the basilar membrane of the guinea pig and on the spiral blood vessels of the tympanic lip of the osseous spiral lamina of the rabbit respectively On the contrary, Spoendlin & Lichtensteiger (1966) and Ross (1971) denied the presence of the adrenergic fibers distributing to these blood vessels in the cat and rat respectively The discrepancy would be due to species difference

In this study, such perivascular sympathetic nerve fibers were also found to be involved in the degeneration process only temporarily This indicates that these fibers are efferent in nature and able to regenerate

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ZUSAMMENFASSUNG

Die Veränderungen der Nervenfasern in dem geschädigten Cortischen Organ des Meerschweinchens wurden ultrastrukturell 3–137 Tage nach perilymphatischem Durchfluß von 20%iger SM Losung studiert Die meisten Nervenfasern zeigten degenerative Veränderungen erst nach 4 Tagen Latenzzeit Jedoch reaktionär vergrößerten sich manche uberlebenden Fasern und entwickelten sich weiter in markhaltige sowie in marklose Fasern durch Umhullung der Schwannschen Zellen die durch Habenula perforata in das Cortische Organ eingedrungen waren Es kamen auch Regeneration und Sprossung der uberlebenden Nervenfasern vor Die regenerierten Fasern erreichten die Reifheit aber atrophierten dann nach 60 Tagen und verschwanden schließlich Die meisten regenerierten Fasern würden die markhaltigen oder marklosen efferenten Fasern sein Retrograde Degeneration findet in beiden afferenten und efferenten Fasern statt In dem weniger beschädigten Cortischen Organ das mit 2%iger SM oder Ringer Lösung durchflossen war, wurde die Invasion der Schwannschen Zellen nicht gefunden

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EXTRA-TYMPANIC ELECTROCOCHLEOGRAPHY

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Abstract A non invasive technique for recording the cochlear action potential in adults without recourse to sedation or local anaesthesia is presented This technique has been assessed in two ways (1) A group of normal subjects was tested to obtain distributions of response amplitude and latency as functions of stimulus intensity (2) A group of patients with Meniere s disease was tested with trans- and extra tympanic electrocochleography to compare the intensity amplitude functions and wave forms obtained from the two methods On the basis of this study the use of extra tympanic electrocochleography as a replacement for the trans tympanic method is discussed

The most widely used method for recording cochlear potentials in man has been developed by the Bordeaux group (Portmann et al 1967) and involves piercing the tympanic membrane with a stainless steel electrode These potentials have also been recorded from extra tympanic positions by a number of workers The first of these (Yoshie et al 1967) used a needle implant in the meatal wall under local anaesthesia and the same technique was employed by Coats & Dickey (1970) Salomon & Elberling (1971), Elberling (1973, 1974) and in later work by the Japanese group (Yoshie 1968, 1971, Yoshie & Ohashi, 1969, Yoshie & Yamaura, 1969) Other workers have recorded from the ear lobe (Sohmer & Feinmesser, 1967, Sohmer et al , 1972), from the mastoid (Spreng & Keidel, 1967) Recordings from the external auditory meatus using surface methods without anaesthesia have been made by two groups Cullen et al (1972) used a chlorided silver wire with the end covered by saline soaked cotton wool which was positioned near the annulus postero-inferiorly and Yoshie (1973) a chlorided silver ball electrode attached postero-superiorly by electrode paste However, no information on the intensity amplitude latency functions or waveforms was reported

MATERIALS AND METHOD

A group of 15 normal hearing subjects between the ages of 18 and 35 were selected at random for extra tympanic testing To investigate the use of this method in sensori neural deafness a group of twenty patients with symptoms of either unilateral or bilateral Meniere's disease were tested with both trans- and extra tympanic methods

The subjects were placed supine on a couch in an electrically and acoustically unscreened room, the background noise level being typically 40 dB in the low frequencies The electrode was coated with electrode jelly and positioned on the annulus, under microscopic control In the mapping experiment three positions were used postero-inferior, postero-superior and anterior The reference electrode was placed on the ear lobe The procedure for

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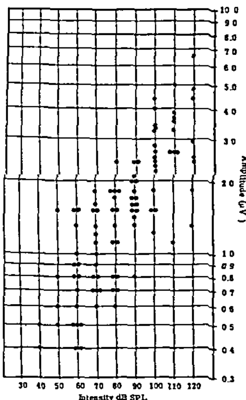


Fig 3 The extra tympanic cochleogram Amplitude intensity relationship in a group of normal subjects

postero-inferiorly and all subjects reported that this was also the most comfortable Therefore this position was used for further testing

Fig 3 shows the amplitude distribution of the action potential (AP) as a function of intensity in 15 normal hearing subjects All gave responses down to 60 dB SPL The ranges of the amplitude values obtained were, 1 3–6 4 μV at 120 dB SPL and 0 4–1 6 μV at 60 dB SPL This compares with 2 μV at 120 dB SPL from a needle implant in the meatal wall (Salomon & Elberling, 1971), 0 5–5 μV at 70 dB SPL (Yoshie et al , 1967) 0 6–2 5 μV at 117 dB SPL and 0 2–0.5 μV at 61 dB SPL from a surface recording in the ear canal (Montandon et al , 1975*a*)

Fig 4 shows the waveform of the AP as a function of intensity and the usual intensity-amplitude latency function in a normal subject

The subjective–objective gap is shown in histogram form in Fig 5 All subjects gave responses within 30 dB of their subjective thresholds, the mean value being 21 3 dB

Fig 6 shows the latency-intensity relationship The mean values obtained were 1 6 msec at 120 dB SPL, 3 3 msec at 60 dB SPL and 4 1 msec at 40 dB SPL

In patients with Meniere's disease

The intensity amplitude latency functions using trans- and extra tympanic electrocochleography from a patient with unilateral

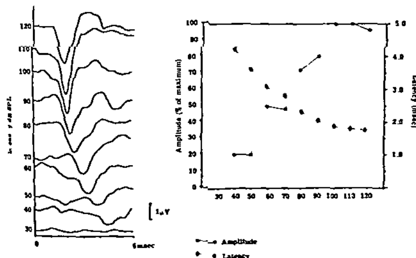


Fig 4 The normal extra tympanic cochleogram. Intensity-amplitude-latency functions

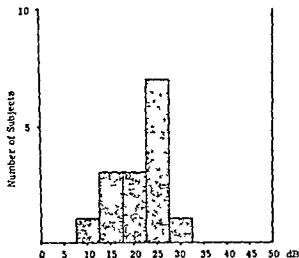


Fig 5 The subjective–objective threshold gap for the extra tympanic cochleogram

Meniere's disease is shown in Fig 7 The similarity in the records was typical

Fig 8 shows the abnormal waveforms recorded from the affected ears in three patients These can be classified as type (i) presence of a pre n_1 negative notch, type (ii) the presence of a pre n_1 positive notch and type (iii) diffuse response with a poorly resolved n_1 and n_2 The polarity of the summating potential was reversed in the extra tympanic responses of the four cases of type (ii) recorded

Finally Fig 9 shows the ratios of the amplitudes of the AP recorded at 100 dB SPL by the two methods in 9 patients who had normal hearing in one ear

DISCUSSION

This method of extra tympanic ECoG can be used as a hearing threshold indicator as all subjects gave responses to within 30 dB of subjective threshold Cullen et al (1972) gave a figure of up to 60 dB for this subjective objective gap while Yoshie (1973) reported a mean value of 17 8 dB However the main use is not in the few cases of non organic hearing loss but rather in the assessment of patients with cochlear pathology

Using trans tympanic ECoG a number of workers have commented on the typical recruiting type intensity amplitude functions of the AP and the abnormal waveforms in a high proportion of patients with Meniere's disease (Portmann et al , 1973, Yoshie 1973, Schmidt et al , 1974) In patients with this disorder the intensity-amplitude functions using the two methods were identical in showing loss of the L curve and in normal hearing subjects the standard H and L curves were present Occasionally, when recording from the meatus patients with non recruiting deafness gave recruiting type functions and similar findings have been reported by Montandon et al (1975*b*) The absence of the L curve in those cases was probably caused by the combination of high muscle activity and low amplitude potentials giving such poor signal to noise ratios that the summated AP could not be detected at the lower intensities Improvement of the ratio can be gained by allowing the patient to lie down quietly before testing as reductions of up to 75% in the mean rms value of the background activity over 30 minutes have been noted Conclusions about the type of hearing loss made from the intensity amplitude curve should therefore be made with caution

The waveform recorded by this extra tympanic method was similar to the wave

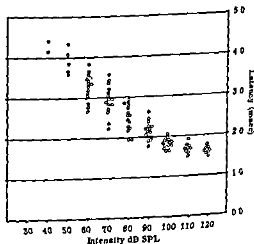


Fig 6 The extra tympanic cochleogram Latency intensity relationship in a group of normal subjects

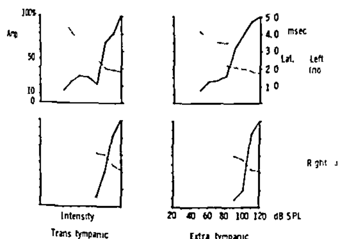


Fig 7 The extra tympanic cochleogram Intensity amplitude latency funct ons of a pat ent with unilateral Meniere s disease

form of the promontary recorded AP Normal subjects showed n_1 and n_2 components at the higher intensities but a diffuse potential at the lower In several subjects the transitional phase, noted by Elberling (1973), in which the dominant n_1 was replaced by a larger n_2 as the intensity decreased, was seen Trans tympanic ECoG in patients with Meniere's disease showed three types of abnormal waveform Types i and ii almost certainly involve the summating potential which is large in this disease, according to Schmidt et al (1974) The extra tympanic recordings were similar to the trans tympanic except in the type ii response, the reversal of the notch polarity may be due to the change in the relative positions of the active electrode and the summating potential generators The relationship between the electrophysiological and clinical findings will be reported elsewhere So far no attempt has been made to record the cochlear microphonic using this technique but two previous reports (Elberling & Salomon, 1973, Yoshie & Yamaura 1969) have described recording of this potential from a needle implant in the meatal wall

It seems likely that the main value of electrocochleography will lie in the study of pathological states of the inner ear and auditory nerve

If progress is to be made in this direction, it will be necessary to collect many recordings

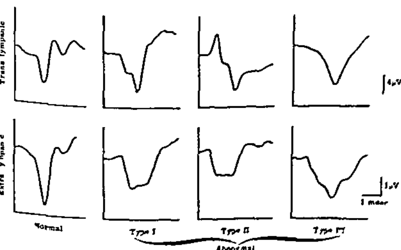


Fig 8 Incidence of abnormal cochleograms in Meniere s d sease

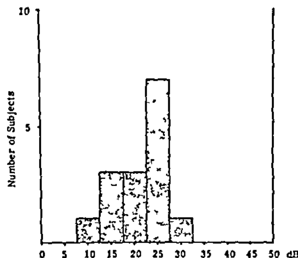


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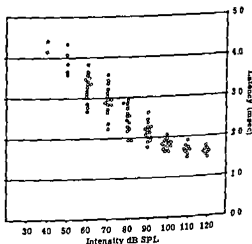


Fig 6 The extra tympanic cochleogram Latency intensity relationship in a group of normal subjects

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DISCRIMINATION OF FREQUENCY RAMPS IN SUBJECTS WITH COCHLEAR HEARING LOSS

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Abstract The auditory sensitivity for detecting linear frequency sweeps of a continuous pure tone has been studied in ten young subjects with cochlear hearing loss The mean thresholds were elevated by a factor of 2 8 as compared with a normal group over the whole range of ramp durations studied (10–500 msec) The results show that this elevation is most likely caused mainly by the cochlear lesion *per se* other possible factors having only a minor effect No clear correlations could be found between thresholds for frequency change and results of other pure tone audiometric tests Such tests thus cannot predict a subject s frequency discrimination

mpaired frequency discrimination as a con quence of sensorineural hearing loss has een reported in some previous investigations In most of these studies pairs of tone pulses, differing in frequency were used as stimuli (Butler & Albrite, 1956, DiCarlo 1962, Ross et al , 1965, Parker et al 1968) The mean values of the difference limen (DL) for frequency in such subjects were found to be larger than in normal subjects by a factor of 1 6 to 7 Meurmann (1954) obtained similar results using stepwise changes in the frequency of a tone as stimulus A subgroup in Meurmann's study, diagnosed as having Meniere's disease, showed higher DL's than subjects with other types of sensorineural hearing loss Konig (1957) noted a clear correlation between frequency discrimination and age in an otologically normal population

This study was supported by grants from the Swedish National Board for Technical Development

In the age range from 60 to 69 years, DL's 3 to 5 times larger than those of young subjects (20–29 years) were obtained in the frequency range 125 Hz–4 kHz Some investigators have found a correlation between frequency discrimination and speech discrimination scores (DiCarlo, 1962, Ross et al , 1965, Gengel 1973)

Frequency modulated (FM) stimuli, whereby tones are modulated either periodically or on a ramp basis (sweep tones), the frequency of the tone varying linearly up or down in the latter case, are similar to many natural sounds (e g formant transitions in human speech) Sergeant & Harris (1962) studying normal hearing subjects, found different DL's for frequency, depending upon the type of sound stimulus used, i e ramps continuous FM and paired frequency-discordant tone pulses They interpreted these discrepancies as evidence for the existence of three different auditory abilities in the general area of pitch Except for the investigations of Meurmann (1954), using continuous rectangular FM on human subjects with sensorineural hearing loss, and of Kelly & Whitfield (1971), using frequency ramps on cats with cortical lesions very little has been done to elucidate auditory discrimination of frequency modulation in pathological cases

In previous reports, we have published our results on normal subjects thresholds for

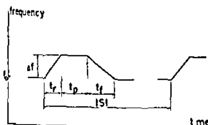


Fig 1 Temporal characteristics of the stimulus

linear frequency changes of a continuous pure tone (Arlinger et al , 1976*a*) as well as on normal subjects slow evoked cortical responses to the same type of stimulus (Arlinger et al 1976*b*) In the work reported here, behavioral thresholds in response to FM-stimuli have been determined in 10 subjects with cochlear lesions An ensuing report will describe recordings of the slow evoked cortical responses to the same FM stimuli in the same subjects We shall also cover some cases with retrocochlear pathology

SUBJECTS

Ten relatively young hearing impaired subjects, diagnosed as having cochlear lesions participated Three of the subjects were men and 7 were women, their ages ranged from 17 to 38 years with a mean of 30 Their pure tone hearing thresholds at 1 kHz (the base frequency used) varied between 25 and 65 dB HL (ISO) with a mean of 48 dB Their audiograms had a relatively flat course around 1 kHz with thresholds at 500 Hz and 2 kHz differing 10 dB or less from the 1 kHz threshold In 7 of the subjects the right ear was tested and in 3 the left ear

The presence of recruitment, as determined by comparing the hearing thresholds with the stapedius reflex thresholds as well as with the Loudness Discomfort Levels (LDL) constituted the diagnostic criterion for cochlear lesions The difference between reflex thresholds and hearing thresholds ranged from 25 to 60 dB with a mean of 44 dB, the difference betwe[illegible]s and hearing thresholds ranged from [illegible]05 dB with a mean of 64 dB Anam[illegible] data speech audiometry, and the [illegible]st when applicable, completed the [illegible] information No findings indicat[illegible] any part of the auditory system other [illegible] the cochlea was involved in any of the su[illegible] hearing loss

Three subjects were diagnosed as having Meniere s disease Their thresholds of hearing were found to remain stable upon repeated checks during the course of the study Five subjects had a congenital, hereditary, bilaterally symmetrical loss four of them with through shaped audiograms and the fifth with a hearing loss sloping slightly towards higher frequencies The hearing loss in one subject was probably genetically determined At the age of 8 this subject had had a small and unilateral loss but now, at the age of 25, he had a moderate and bilaterally symmetrical impairment Finally, one subject s bilateral loss was most likely caused by scarlatina at the age of 4

STIMULUS

The stimulus consisted of a continuous pure tone characterized by its base frequency, f_0 (see Fig 1), which was kept constant at 1 kHz, and its sound level The tone was modulated as to frequency with randomly varied interstimulus intervals (ISI), while its level was kept constant The ISI was varied randomly from 2 sec and upwards with a mean of 4 sec The linear frequency ramp was characterized by its magnitude (Δf) and its duration (t_r) When the ramp had ended the signal remained at the final frequency of the ramp during a certain time (t_p) before returning to the base frequency during t_f The sum of t_r and t_p was kept constant at 500 msec, and t_f was always 600 msec

A special stimulus generator, described in greater detail in a previous report (Arlinger et al 1976*a*), was utilized It has six channels with independent choice of Δf and temporal

stimulus parameters The latter were set to the same values for all channels for each test, different, evenly spaced Δf-values were chosen for the six channels in order to cover the discrimination curve as indicated by preliminary tests as well as possible In each test session, 20 stimuli were presented from each channel, and the order of triggering among the six channels was randomized by means of an electronic random channel selector The stimulus was always presented at the subject's most comfortable level, which ranged from 60 to 90 dB HL (mean 75 dB) This corresponded to sensation levels in the range 11–46 dB (mean 28 dB) All harmonics of the sound signal were 40 dB or more below the fundamental, as measured acoustically in a 6 cc-coupler

The subject responded to a perceived stimulus by pressing a switch The subject's response was accepted when it was made within 1 sec after the onset of the frequency ramp, one count was tallied in the response counter for the channel that was activated The number of accepted responses for each channel was read on a digital display at the end of each test series From these data a psychometric function could then be plotted nd a threshold value calculated

The response scores at very small sub threshold ramps were always less than 10%, i e a low rate of false positive responses This finding, along with the fact that the ISI's varied randomly led us to define threshold as the magnitude of frequency change giving a 50% discrimination score The threshold was calculated by means of iterative linear interpolation (Lance 1960)

EXPERIMENTAL PROCEDURE

During the experimental sessions the subjects were seated in a sound insulated, anechoic chamber, monitored by intercommunication as well as closed circuit TV systems The test sounds were presented monaurally by a head phone (Telephonics TDH 39 fitted with circumaural cushion)

All subjects were introduced to the test situation through a number of training sessions As soon as the subjects showed a stable and reproducible performance, test sessions were started

Each test session began with a standard test in order to follow each subject's performance from session to session At this standard test, ramps with a duration of 10 msec were used The influence of the ramp duration on thresholds was determined at durations of 20 100 and 500 msec Only positive frequency ramps (increasing frequency) were studied since the results from subjects with normal hearing showed no difference between positive and negative ramps In the opening standard test, 10 stimuli per channel were presented, and in the test proper 20 stimuli per channel Thus, after the introductory training sessions, each subject participated in three test sessions on separate days, each session requiring a total testing time of 12–15 minutes The three different ramp durations studied were assigned to the three test sessions in random order for each subject

RESULTS

The mean threshold for frequency change in the three standard tests for the 10 subjects was 7 9 Hz The range of individual means was from 3 8 to 14 0 Hz with a standard deviation of 2 97 Hz (38% of the mean for the group) The mean of the individual standard deviations was 18%

Fig 2 shows the mean values with 1 S D indicated for the thresholds for frequency change at the three ramp durations 20 100, 500 msec Results of the 10 msec ramp duration standard test are also shown The corresponding results from the 10 subjects with normal hearing reported previously (Arlinger et al 1976*a*) are indicated by filled circles and dashed lines The group with cochlear hearing loss showed a statistically significant elevation of the threshold for all ramp durations compared with the normal group ($p<0.05$%

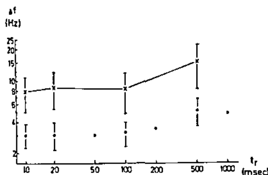


Fig 2 Mean values ±1 S D of the thresholds for frequency ramps as a function of the duration of the ramp in the pathological group (×) The results for the normal group are also plotted (●)

Student's *t*-test) In both groups the threshold at ramp durations 10, 20 and 100 msec is almost independent of ramp duration and statistically significantly lower than at 500 msec ($p<1\%$) The ratio between mean threshold values of the cochlear group and of the normal group was thus approximately constant with values between 2 62 and 2 98 and a mean of 2 8

There is a positive correlation between the results at different ramp durations for each subject, as is shown by ranking the 10 subjects according to their thresholds for frequency change in the standard test and at the three ramp durations tested In the results from the ten subjects, Spearman s coefficients of rank correlation were significantly above zero in all six combinations of ramp durations (confidence level 1% in three combinations 5% in two, and 10% in one combination), thus, a subject who showed a low threshold value for one ramp duration generally did so for the others as well There are consistent differences in performance among the 10 subjects regardless of ramp duration There was however, no significant correlation or rank correlation between the thresholds for frequency change and hearing thresholds stapedius reflex thresholds or loudness discomfort levels (LDL) Nor was any correlation found between thresholds for frequency change and stapedius reflex threshold minus hearing threshold, and LDL minus hearing threshold Nor was there any significant correlation between the sound level used, when that was expressed as sensation level, and thresholds for frequency change When the sound level was expressed as hearing level, i e the subjects' most comfortable levels in dB HL, there was, however, a weak correlation between that level and threshold for frequency change This correlation was significant only at the 2 5% level for the thresholds for the 20 msec ramps, and on the 5% level for the standard stimulus and the 100 msec ramps, it was not significant ($p>10\%$) for the 500 msec ramps

No relation was found between the etiology of the cochlear lesion and the subjects' rankings in the frequency discrimination tests the three Menierecases all placed in the middle of the group (average ranks 4, 5, and 6), and the best and the poorest subjects were both diagnosed as having hereditary losses with trough-shaped audiograms The pure tone hearing thresholds of these 2 patients at 1 kHz differed by only 10 dB

DISCUSSION

The results of the present study show that subjects with cochlear impairment have a significantly higher threshold for frequency change than do subjects with normal hearing Quantitatively, the subjects with cochlear hearing loss showed larger inter- and intra-subject variations than did the normal group As with the normal subjects, the inter subject variation was clearly greater than the intra-subject variation

It is important to determine whether factors other than cochlear damage contribute to the difference in the two groups' thresholds for frequency change One such factor may be age Konig (1957), using pulse pairs, found a clear deterioration of frequency discrimination with increasing age The mean ages of patients and normal group are 30 and respectively By interpolating Köni

at 1 kHz, this age difference could account for a threshold increase on the order of 25% in the older group over the younger one Thus, this age difference can only contribute insignificantly to the difference in thresholds found in the present study

Regarding the influence of sound level on the thresholds for frequency change, our results on 3 normal subjects tested at 20, 40, 60, and 80 dB showed that a sound level below 40 dB HL was required to produce a significant effect on thresholds for frequency change (Arlinger et al , 1976*a*) On the normal subjects, whose sensation and hearing levels were approximately identical, 80 dB did not elicit thresholds for frequency change significantly different from those obtained at 60 dB At 20 and 40 dB the mean thresholds were 80 and 35% higher, respectively, than at 60 dB In the present study a mean signal level of 75 dB HL or 28 dB SL was used, resulting in thresholds 180% higher on the average than those of the normal group at 60 dB The lower sensation levels used might thus be responsible for as much as 50–60% of the 180% increase, the effect is probably smaller, however, since loudness level is probably more portant to the discrimination than sensation vel The effect would be insignificant were the sound level in dB HL considered In this context, hearing level is often a more relevant measure of sound intensity than is sensation level This is further supported by the fact that, in the present study, hearing level showed a significant (though weak) correlation with the individual thresholds for frequency change, whereas sensation level showed none The effect, then of sound level differences on the thresholds for frequency change in normal cases and in cases with cochlear lesions can at most explain only a minor part of the threshold difference

Several previous studies on frequency discrimination have shown a correlation between this auditory capacity and results from other audiometric tests using pure tones of constant frequency as stimuli Meurmann (1954) noted an increased difference limen for frequency with greater degrees of hearing impairment (presumably as indicated by pure tone hearing thresholds) Ross et al (1965) found a similar general trend but having a low correlation coefficient Gengel (1969) found a moderate correlation between threshold of hearing and difference limen for frequency at 500, but not at 250 Hz in a group of 44 children In the present study, a weak positive correlation was found between thresholds for frequency change and the most comfortable level expressed in dB HL Speech discrimination could not be adequately evaluated in regard to correlation with frequency discrimination in the present study, since loss of speech discrimination in quiet was small for all our subjects

The finding that the ability to detect linear frequency ramps of a pure tone is significantly affected by cochlear hearing loss agrees with the findings of previous investigations which have used pulse pairs as well as continuous FM The particularly poor performance of patients with Meniere s disease in Meurmann's study (1954), however, could not be reproduced in our group These results do show that frequency discrimination is disturbed by cochlear hearing loss, regardless of the particular type of stimulus being used

The results of this study support the previously proposed model (Arlinger et al 1976*a*) of perception of frequency ramps for subjects with normal hearing This model assumes a common mechanism for the whole range of ramp durations The detection threshold for short ramp durations (<200 msec) is determined mainly by the magnitude of the frequency change (Δf), while at long ramp durations (>200 msec), a slope detection function dominates This hypothesis has been further developed in a second paper (Arlinger et al , 1976*b*), where slow evoked cortical responses to frequency ramps were studied In that report, a functional model was suggested which involved integration of the time derivative of the signal frequency, i e the rate of

frequency change Assuming an adaptable integration time, determined by the rate of frequency change, the result of the integration will be determined by the magnitude of the frequency change if the ramp is of shorter duration than the integration time corresponding to the rate of frequency change If, on the other hand, the ramp duration exceeds the integration time, the result of the integration will be determined by the slope of the ramp This model implies that the auditory system uses essentially the same mechanisms for detection of frequency ramps regardless of their duration The present study indicates that this model is well fitted to the pathological group having thresholds elevated by a constant factor over the whole range of durations studied

The actual frequency discrimination and the prior data processing occur in parts of the auditory nervous system more central than the cochlea The fact that the ability to detect a difference or change in the frequency of an acoustical signal is significantly altered by a presumably peripheral lesion in the cochlea does, however, raise some interesting and baffling questions For example is the change in difference limen for frequency related to a change in the tuning curves of the basilar membrane or to some other measure of the physiological properties of the auditory periphery? One might also ask to what extent impaired frequency discrimination is related to the degree of loss of hair cells in the cochlea

CONCLUSIONS

Discrimination of frequency ramps of a continuous pure tone has been studied in 10 subjects with hearing loss of cochlear origin The following conclusions can be drawn

(1) Frequency discrimination is significantly impaired compared with that of normal subjects, with thresholds for frequency change increased on the average by a factor of 2 8, regardless of the duration of the frequency sweeps in the range 10–500 msec

(2) This threshold elevation is most likely caused by the cochlear lesion *per se* Differences in age and sound level between normal and pathological groups can explain only a minor part of this elevation

(3) Thresholds for frequency change could not be predicted on the basis of audiometric tests using pure tones of constant frequency as stimuli

(4) The idea of a common mechanism for the detection of frequency differences and of frequency changes in a continuous pure tone has been supported

ZUSAMMENFASSUNG

Die Frequenzunterschiedsschwellen für 10 Versuchspersonen mit Innenohrschwerhorigkeit sind untersucht worden Lineare Frequenzrampen eines Dauertons wurden als Reiz benutzt Die durchschnittliche Schwellen waren mit einem Faktor von 2 8 erhoht im Vergleich mit den Schwellen einer normaler Gruppe Die Resultate zeigen dass diese Erhöhung wahrscheinlich im Innenohr schaden an sich seinen Grund hat Andere mogliche Ursachen können die Resultate nicht erklaren

Keine klare Korrelation wurde zwischen Frequentz unterschiedsschwellen und die Resultate von anderen audiometrischen Untersuchungen gefunden Solche Untersuchungen konnen also nicht die Frequenzdiskrimination eines Patienten voraussagen

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THRESHOLDS FOR LINEAR FREQUENCY RAMPS OF A CONTINUOUS PURE TONE

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Abstract The human auditory sensitivity in detecting linear frequency ramps of a continuous pure tone has been studied It is shown that for short ramp durations (<200 msec) discrimination depends on the difference between base and plateau frequencies the mean threshold being about 3 Hz at 1 kHz For longer ramp durations (>200 msec) discrimination was found to be based on detection of the actual frequency sweep No significant difference was found between thresholds for upward and downward sweeps Expressed in Hz the threshold for frequency change was approximately constant for base frequencies up to 1 kHz above which it increased reaching approximately 14 Hz at 4 kHz There was no significant difference in the threshold for frequency change from 40 to 80 dB HL but at 20 dB HL the threshold was significantly higher than at 40 dB HL Intra individual variation in thresholds was found to be smaller than inter individual variation The results are discussed in relation to previous frequency discrimination data where either tone pulse pairs continuous frequency modulation or frequency ramps were used as stimuli

Man's ability to detect differences and changes in frequency of an auditory signal has been the subject of several studies in normal as well as pathological cases

Basically, three types of stimuli in the frequency domain have been used Pairs of tone pulses with different frequencies (e g Harris, 1952, Konig, 1957 Nordmark, 1968, Moore, 1973), periodic frequency modulation (FM) of various modulating frequencies and wave forms (e g Shower & Biddulph, 1931, Zwicker & Feldtkeller, 1967 Kay & Matthews, 1972), or non periodic FM mainly linear frequency ramps (e g Sergeant & Harris, 1962, Pollack 1968, Nabelek & Hirsh, 1969, Tsumura et al, 1973)

This study was supported by grants from the Swedish National Board for Technical Development

Sergeant & Harris (1962) discussed the possible correlation between thresholds for these different stimuli Generally finding low correlation coefficients, they concluded that "three different auditory abilities in the general area of pitch" were at work

Summarizing these studies, it can be observed that frequency discrimination is influenced by the way the stimuli are presented regardless of type For the discrimination of frequency ramps, the total frequency change as well as rate of frequency change seems to be of importance The psycho physical method employed in a particular experiment may also influence the results obtained The extent to which common mechanisms are involved in detecting frequency difference, periodic FM and non periodic frequency ramps is still unclear

Purpose of present study

Frequency discrimination may be influenced by pathological conditions in the auditory system (e g Meurmann, 1954, Butler & Albrite, 1956, Konig, 1957, Ross et al, 1965, Brandt, 1967, Parker et al, 1968, Campbell, 1970, Danaher et al, 1973) Obtaining a reliable measure of a patient's ability to discriminate frequency difference or change thus has a potential clinical value One reason that clinical measurement of frequency discrimina

tion is seldom carried out is quite likely the patients' reluctance to invest the training time necessary if psycho physical methods are to yield reliable results

An electrophysiological method could be an alternative to psycho physical methods The best electrical response seems to be the slow evoked cortical response, since only sounds with very rapid changes produce recordable electrophysiological signals peripherally in the auditory system Studies on the cortical response to stimuli in the form of frequency ramps have been reported by e g Spoor et al (1969), Ruhm (1970) and Lenhardt (1971)

If clearly related to the psycho physically determined frequency discrimination, the evoked response can be recorded and take on the role of a valuable clinical tool for determining abnormalities in this discrimination This is of importance both for diagnostic purposes and for evaluating the possible benefit of e g frequency transposition or frequency compression Evoked responses might also be used to differentiate between vibratory and auditory perception in patients with severe hearing impairment, since vibratory frequency discrimination is assumed to be considerably poorer than auditory (Pickett & Martony, 1970)

The present paper describes the results of a systematic study of behavioral thresholds for linear frequency ramps of a continuous pure tone The results of recording the slow evoked cortical response with the same type of stimulus (using supra threshold ramps) from the same subjects, all of whom had normal hearing, will be reported in a later paper The results of behavioral and electrical response studies on subjects with impaired hearing will also be presented later

SUBJECTS

Ten young subjects with normal hearing were studied Their ages ranged from 23 to 30 years with a mean of 25 Their thresholds of hearing for the frequencies examined (125 Hz–8 kHz) were 10 dB HL (ISO) or less with a mean of 1 dB HL Their stapedius reflex thresholds (at 250 Hz–4 kHz) were in the range 75 to 90 dB HL with a mean of 86 dB None of the subjects was a trained listener, musician or singer

The stimulus was always presented monaurally to the left ear using a Telephonics TDH 39 ear phone fitted with a circumaural cushion Subjects were seated in a sound insulated anechoic chamber, monitored by intercom as well as closed circuit TV systems The subject indicated each perceived stimulus by pressing a microswitch held in the hand

All subjects were introduced to the test situation through a number of training sessions As soon as a subject arrived at a stable and reproducible performance, test sessions were started Each test session was begun with a standard test in order to follow each subject s performance from session to session to detect possible long term learning effects etc

STIMULI

The stimulus consisted of a continuous pure tone, characterized by its base frequency, f_0 (see Fig 1), and its level, expressed in dB HL While its level was kept constant the tone was modulated as to frequency with randomly varied interstimulus intervals (ISI) The linear frequency change was characterized by its magnitude (Δf) and its duration (t_r) After the end of the ramp the signal remained at the final frequency of the ramp for a certain time (t_p) before returning to the base frequency during time t_f The sum of t_r and t_p was 500 msec when studying t_r values up to 500 msec For t_r=500 msec, a (t_r+t_p) value of 1 000 msec was also used, and for t_r=1000 msec, (t_r+t_p) values of 1 000 and 2 000 msec were used The fall time, t_f, was kept constant at 600 msec Both upward and downward ramps were studied

The generator for the stimulus described in detail previously (Arlinger et al , 1975) con

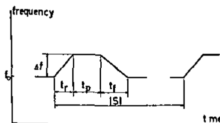


Fig 1 The temporal parameters of the stimulus

sisted of a trigger source and six parallel identical pulse forming channels, controlling a voltage-controlled oscillator (VCO) (see Fig 2) The sinusoidal output signal of the VCO was attenuated, amplified and fed to the ear phone

The trigger source generates short impulses, each of which initiates a stimulus The randomly varying ISI's occur with a probability density function of an exponential type The attainable ISI values are limited on the lower side but not upwards In this study, the minimum ISI was set to 2 sec for all t_r-values except for 1 sec, when it was increased to 3 sec A standard deviation of 2 sec was used The mean ISI, which equals the sum of the minimum and the standard deviation (Arlinger, 1969), was thus 4 sec for all t_r values except 1 000 msec, when it was 5 sec

Each trigger impulse activates one of the six parallel pulse forming channels, as determined by a random channel selection function On each of the six channels the values of t_r, t_f, magnitude and direction of the frequency change can be selected The pulse forming in the six channels was set differently giving evenly spaced Δf values in steps of 0 05 or 0 1% This was done in order to obtain scores covering the steep part of the response probability function

The total harmonic distortion of the sound was −40 dB or less as measured acoustically in a 6 cc-coupler This degree of sound purity was sufficient except at the two lowest base frequencies studied (250 and 500 Hz) For those frequencies the harmonic distortion was reduced further to −60 dB or less by means of a band pass filter (Krohn Hite 3750) For 250 Hz base frequency the filter had −3 dB limits at 180 and 300 Hz, and for 500 Hz it had 3 dB limits at 380 and 600 Hz This gave 0 5 dB attenuation at 220/290 and 440/560 Hz re centre frequency, respectively The roll-off of the filter curves was 24 dB/octave Consider a tone frequency modulated with a triangular signal (equivalent to $t_r=t_f$, $t_p=0$) The main energy of the modulating signal is below $f_m = 1/t_r$ The ramp duration used in the present study, $t_r=50$ msec, gives $f_m=20$ Hz The largest frequency sweep used on any subject at tests at 250 and 500 Hz was 6 Hz These values give a modulation index of 0 3, i e narrowband FM, which requires a bandwidth approximately equal to the bandwidth of the modulating signal for faithful reproduction (Schwartz, 1959) Thus, the filter bandwidths were sufficiently large to avoid distorting the signal

The subject responded to a perceived stimulus by pressing a switch The response was accepted when it was made within a certain time after the onset of the frequency ramp, and one count was tallied in the response counter for the channel that was activated The response time allotted was 1 sec, except for $t_r=1$ sec, in which case it was increased to 2 sec in order to allow a realistic reaction time after the end of the ramp

The number of accepted responses for each channel was read on a digital display at the

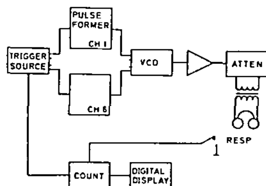


Fig 2 Schematic diagram of the stimulus generator

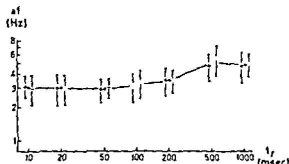


Fig 3 Mean values of absolute thresholds for positive (+) and negative (−) ramps ±1 S D as a function of ramp duration The line connects the averages of the means for positive and negative deviations

end of each test series A psychometric function could then be plotted and a threshold value calculated

The response scores at very small subthreshold ramps were in the range 0–10% i e indicating a low rate of false positive responses This finding along with the fact that ISI s were varied randomly led us to define threshold as the magnitude of the frequency change giving a 50% discrimination score The threshold was calculated by means of iterative linear interpolation (Lance 1960)

EXPERIMENTAL PROCEDURE

Each session started with a standard test with ramp duration $t_r = 10$ msec and upward sweep

Each of the 10 subjects was studied during fourteen test sessions on different days and faced with several different ramp durations from 10 to 1000 msec in 1–2–5 steps Both upward and downward sweeps with a base frequency of 1000 Hz and a signal level of 60 dB HL were used

Twenty stimuli were presented from each channel in the test proper giving a total of 120 stimuli from six channels This set up with a mean ISI of 4 sec gave a mean duration of 8 min for each test The different sets of stimuli for a given test were chosen randomly

For the standard test preceding each test 10 stimuli per channel were presented requiring a mean standard test duration of 4 min

Two subgroups of 3 subjects each were studied further with regard to influence of base frequency and sound level respectively Base frequencies of 250 500 1000 2000 and 4000 Hz were used at a sound level of 60 dB HL and a ramp duration of 50 msec For 1000 Hz base frequency signal levels of 20 40 60 and 80 dB HL were used also with ramp duration 50 msec Both upward and downward sweeps were studied

In addition one subject was studied in detail with regard to the influence of the plateau time, t_p, on the thresholds for frequency ramps

RESULTS

A *Standard test (1000 Hz 60 dB HL 10 msec positive ramps)*

The mean threshold for frequency change obtained from the fourteen standard tests for all 10 subjects was 2 78 Hz The range of individual means was from 1 64 to 3 28 Hz with a standard deviation of 0 65 Hz (23% of the mean for the group) The individual standard deviations ranged from 9 to 17% with a mean of 13%

B *Influence of ramp duration and direction of frequency change (1000 Hz 60 dB HL)*

In Fig 3 are shown the thresholds (in Hz) of the group as a function of ramp duration and direction of the frequency change Mean values ±1 S D are given No statistically significant difference between thresholds for upward and downward sweeps was seen at any of the ramp durations studied (Student s *t* test $p > 10\%$)

At ramp durations below 100–200 msec the threshold remains constant irrespective of ramp duration Above 200 msec ramp duration the threshold increases with increasing ramp duration The thresholds at ramp durations of 500 and 1000 msec (upward and downward threshold pooled) are significantly higher

Table I *Means and standard deviations for ' absolute ' and normalized thresholds as a function of duration (t_r) and direction of the frequency ramp*
Base frequency 1 kHz signal level 60 dB HL 10 subjects

t_r (msec)	Dir	Abs threshold(Hz) Mean	S D	Norm threshold Mean	S D
10	pos	3 07	0 67	1 11	0 10
10	neg	2 98	0 90	1 06	0 17
20	pos	3 06	0 96	1 10	0 23
20	neg	3 02	0 91	1 07	0 14
50	pos	2 91	0 70	1 05	0 07
50	neg	3 05	0 66	1 11	0 12
100	pos	3 06	0 89	1 09	0 17
100	neg	3 33	0 92	1 20	0 15
200	pos	3 50	0 99	1 26	0 18
200	neg	3 52	0 86	1 27	0 14
500	pos	4 79	1 35	1 76	0 40
500	neg	5 64	1 75	2 02	0 32
1 000	pos	4 73	1 39	1 72	0 35
1 000	neg	4 98	1 21	1 83	0 38

than the thresholds at 200 msec and less ($p<0.05\%$)

With regard to the individual data, it was found that a subject who showed a low threshold value for a given ramp duration generally did so for the other ramp durations and for the standard stimulus as well The Spearman coefficient of rank correlation, (R) was calculated in order to evaluate this tendency For the 21 possible combinations of ramp durations, the values of R ranged from 0 36 to 0 98 with a mean of 0 70 In 13 of the combinations the rank correlation was statistically significant, in 4 of these at the 0 1%-level and in 5 at the 1%-level Therefore, in order to emphasize the influence of ramp duration a normalized threshold for each ramp duration in each individual subject was calculated The normalized threshold was defined as the ratio between each subject's threshold at a given combination of test stimulus parameters and his/her mean threshold for the standard stimulus Table I presents both absolute (in Hz) and normalized mean thresholds together with their standard deviations The degree of statistical significance of the difference between the normalized thresholds at 500 and 1000 msec and the normalized thresholds at 200 msec ramp duration increases as compared with the absolute values of the thresholds (t-values are 6 83 and 5 79 as compared to 4 18 and 3 85 for absolute thresholds, $p<0.05\%$)

The results of using different plateau durations, t_p, are shown in Fig 4 for one subject For short ramp durations, a shortening of the plateau causes a clear increase in the threshold for frequency change For longer ramp durations, at and above 200 msec, t_p does not show any influence on the threshold for frequency change

C *Influence of base frequency (60 dB HL 50 msec ramp duration)*

Fig 5 shows the means of the absolute values of the threshold and of the relative frequency

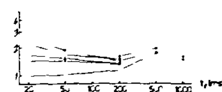


Fig 4 Normalized (re t_r=50 msec t_p=450 msec t_f=500 msec) thresholds for one subject as a function of t_r. Plateau durations used are t_p=0 (×) 5 msec (○) 10 msec (□) 20 msec (△) 50 msec (▽) and ≥300 msec (●) For t_p ≥300 msec t_f was 500 msec otherwise t_f=t_r. Base frequency 1 kHz 60 dB HL.

thresholds, i e the ratio between frequency change and base frequency The values for upward and downward sweeps were averaged for the 3 subjects studied in this regard Table II presents the absolute mean values and the relative mean values with their standard deviations The differences in the absolute values of the thresholds are highly significant ($p<0\ 05\%$) at 1 and 2 kHz, as they are at 2 and 4 kHz The differences between the absolute values of the thresholds at 250, 500 and 1 000 Hz are not significant ($p>10\%$) For the relative thresholds all pairs of mean values at *neighboring frequencies are significantly* different

D *Influence of sound level (1 kHz 50 msec ramp duration)*

The means of the absolute value of the thresholds were found to be a function of signal level (Fig 6) in the 3 subjects studied in this regard Table III presents the absolute thresholds and the normalized thresholds expressed as mean values and standard deviations

The normalized thresholds at 20 dB HL are significantly higher than those obtained at 40, 60 and 80 dB ($p<5\%$, 0 5% and 0 5% respectively) Thresholds at 40 dB HL are significantly higher than those at 60 dB ($p<5\%$)

DISCUSSION

Standard test

The results of the standard test were utilized to assess whether any learning effects arose as well as to compare inter and intra individual variation The behavioural threshold for the standard stimulus showed no consistent trend in any of the 10 subjects indicating that *after the introductory training sessions* no significant learning effects occurred during the entire testing period The variations were random around a constant mean value

Since the interindividual variation was clearly larger than the intra individual variation and since the rank order correlation between the subjects was significant for all ramp

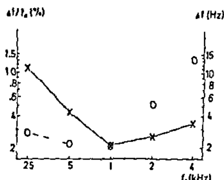


Fig 5 Mean values of absolute (O—O) and relative (×—×) thresholds as a function of base frequency

*durations it seems justified to take the normalized thresholds for each subject, calculated as described above, for evaluating the influence of various stimulus parameters In this way the effects on thresholds of varying the different stimulus parameters are less obscured by inter subject variation

Ramp duration and direction

The results of the present study show that the thresholds for frequency change are independent of the duration of the frequency ramp as long as durations are short (<200 msec) These thresholds then attain a maximum value around 500 msec and become somewhat though not significantly, lower at 1 000 msec duration This rather irregular relation, as shown in Fig 3 suggests that the relative importance of different parameters of

Table II *Means and standard deviations for absolute and relative thresholds as a function of base frequency*

Thresholds for positive and negative ramps pooled Signal level 60 dB HL t =50 msec 3 subjects

Base freq (Hz)	Thresholds Abs (Hz) Mean	S D	Rel (%) Mean	S D
250	2 79	0 88	1 116	0.351
500	2 19	0 36	0 438	0 071
1 000	2 12	0 36	0 212	0 036
2 000	5 15	0 98	0 258	0 049
4 000	13 82	2 17	0 346	0 054

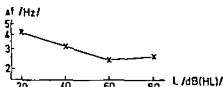


Fig 6 Mean values of absolute thresholds as a function of sound level

the stimulus for the detection of a frequency change depends upon the duration of the frequency ramps

Three different parts of the stimulus could influence the detection of frequency change separately, namely the initial ramp of duration t_r, the plateau of duration t_p, and the return to the base frequency during t_f. If the discrimination of frequency change were based upon the upward and downward ramps entirely, one would assume that ramp duration, t_r, and possibly also t_f, would influence the threshold. Similarly, if detection of a frequency change were due to the presence of a plateau, plateau duration, t_p, would then be an important parameter. The results shown in Fig 4 seem to resolve this question to a great extent

These results show that for short ramp durations the duration of the plateau, t_p, has a considerable influence on thresholds. They also show that for short plateau durations, the durations of the frequency ramps have considerable influence over the whole range studied (10–1 000 msec). These findings suggest that the ear uses the difference between base and plateau frequencies, the total frequency change, for detection purposes when ramp durations are short. However, when the plateau is very short or non existent, the detection of the actual ramp may play a role even at short transition durations. This is also supported by comparing the data from Fig 4 of the present study with results from the studies by Sergeant & Harris (1962) and Tsumura et al (1973) using frequency ramps. Some of the results of these two studies are shown in Fig 7, together with our data. With an initial frequency of 1 000 Hz, Tsumura et al used 1 sec long tone pulses and kept the duration of the initial steady frequency segment at 500 msec. The ramp duration was varied from 5 to 300 msec, and the final steady frequency segment thus obtained a duration of 495 to 200 msec. This stimulus configuration resulted in very little influence of ramp duration on the thresholds. When the initial steady segment was reduced in duration (to e g 30 msec) they found that discriminability of the ramps became substantially poorer with decreasing ramp duration. Sergeant & Harris found that ramp duration exerted a very strong influence on the total frequency change necessary for discrimination. The threshold had a pronounced minimum value at a ramp duration of 2 sec. These findings agree qualitatively with our data. Taken together, these results suggest that the auditory detection of frequency ramps may be based either on detecting frequency difference or on detecting the actual frequency sweep. Which of the two functions is the more important for the detection of a certain type of stimulus depends on the duration of the frequency sweep

The results obtained in the present study for long ramp durations remain to be explained. Figure 4 shows that the plateau duration in one subject has no influence on the thresholds for frequency change for ramp durations of 500 and 1 000 msec. This has been confirmed on three other subjects. Three sub-

Table III *Means and standard deviations for "absolute" and normalized thresholds as a function of sound level*

Thresholds for positive and negative ramps pooled. Base frequency 1 kHz t_r=50 msec. 3 subjects

Level (dB HL)	Thresholds "Abs" (Hz) Mean	S D	Norm Mean	S D
20	4 23	0 61	1 93	0 56
40	3 19	1 51	1 34	0 37
60	2 38	0 77	1 02	0 11
80	2 56	1 01	1 08	0 14

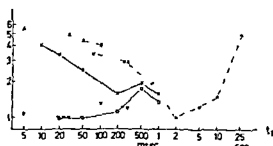


Fig 7 Normalized thresholds for the same subject as in Fig 4 for $t_p=0$ (×) and $t_p \geq 300$ msec (O) Also shown are normalized thresholds from Sergeant & Harris (1962 re ramp duration 2 sec) (□) and from Tsumura et al (1973 re initial steady segment duration $T_1=500$ msec and ramp duration $T_2=30$ msec) for initial steady segment duration $T_1=30$ msec (Δ) and $T_1=500$ msec (∇) Abscissa is ramp duration t_r

jects were shown to respond immediately, on the average, at the very end of the ramp when durations were 500 and 1 000 msec, provided the frequency change was just above threshold At changes twice the individual threshold values, the subjects responded clearly before the end of the ramps (Response was defined as the actual response time minus the time for response at large supthreshold stimulation about 200 msec)

Both these findings show that the subjects perceive the actual frequency sweep at these long ramp durations The increase in threshold with ramp duration in the range 200–500 msec might be explained in terms of interaction between the two detection functions described above For long plateau durations and short ramp duration the subject detects the frequency difference between base and plateau At longer ramp duration, the increasing possibility of detecting the actual ramp may interfere with the detection of frequency difference This theory receives support from Elliott s (1971) finding that frequency discrimination for tone pulse pairs was poorer when the interval between them consisted of tones than when it consisted of silence The detection of frequency difference would consequently deteriorate until the detection of the actual frequency sweep becomes the more sensitive function and determines the threshold That seems to occur around 500 msec ramp duration

Another possible explanation, in different terms, presumes a mechanism which integrates the rate of frequency change of the signal with an integration time (T) of the order of 200 msec When the ramp duration is less than T, the result of the integration is proportional to the total frequency change When the ramp duration exceeds T, the result will be proportional to the slope of the ramp Thus a larger frequency change is required in order to reach a certain level at the integrator output This simple model could explain the increase in thresholds in the ramp duration range from 200 to 500 msec However, in order to explain why the thresholds at 1 000 msec ramp duration are approximately the same as those at 500 msec, an additional mechanism is required, parallel with the integrator The threshold value for the integrator channel is lower than that of this parallel channel, when ramp durations are less than 500 msec At 500 and 1 000 msec ramp duration, however, this parallel channel becomes the more sensitive one and determines the detection of the ramp

Pearson's correlation coefficient for the individual thresholds for frequency change for a short ramp duration ($t_r=50$ msec) and for a long one ($t_r=500$ msec), is 0 80 when the average threshold for positive and negative ramps is used This is significantly greater than zero (1% level of significance) Thus, if thresholds at $t_r=50$ msec represent detection of the frequency difference and at 500 msec detection of the actual frequency sweep, a statistically significant correlation exists between the sensitivities of these two functions In this connection it may be noted that Sergeant & Harris (1962) found a significant, albeit small correlation between 'pitch memory' and "recognition of glissando' These results suggest that these functions rely at least partly on common physiological mechanisms

In short, detection of frequency change of a tone with the stimulus configuration used in

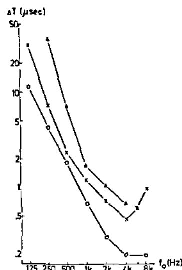


Fig 8 Least discriminable time interval $\Delta T = \Delta f/f^2$ as a function of base frequency Our results (Δ) are shown together with those of Moore (×) (1973 subject T C tone pulse duration 200 msec 60 phones) and those of Nordmark (O) (1968 6 subjects 45 dB SL)

our main tests, with long t_p and t_r-values, is concluded to be based mainly on the frequency difference at short ramp durations (<200 msec) and on the changing frequency at long durations (>200 msec) Thus, by choosing the proper value of t_r either function can be studied in separation or in combination

The direction of the frequency change had no significant influence on the thresholds for any stimulus configuration used This is in agreement with the findings of several previous investigations on frequency discrimination, using pairs of tone pulses (Harris, 1952, König, 1957) and frequency ramps (Nabelek & Hirsh, 1969) On the contrary, Tsumura et al (1973) observed that their two subjects tended to have greater thresholds for rising than for falling frequency ramps

Base frequency

Our results (Fig 5) differ somewhat from previously published data with regard to the lower frequency range (e g König, 1957, Nordmark, 1968, Moore 1973) Regardless of whether tone pulse pairs continuous frequency modulation or frequency ramps were used, the absolute values of the thresholds for frequency difference or change tended to decrease with decreasing base frequency, also below 1 kHz, in their studies, while ours remain approximately constant

As mentioned above we used a band pass filter to suppress the harmonics of the signal at base frequencies 250 and 500 Hz According to the discussion in the instrumentation section above, the filter can hardly have had any influence on the detection of changes in the fundamental frequency of the signal Different degrees of harmonic distortion may still be a possible explanation for the difference in thresholds between our study and other investigations using low base frequencies Also the different types of stimuli used in the different investigations may not have the same base frequency dependence

Replotting our data as difference in time interval necessary for discrimination, as suggested by Nordmark (1968, 1970), in logarithmic coordinates gives the result shown in Fig 8 Just as was the case for Nordmark's and other investigators' data, our results can be fitted with two straight lines with a change of slope in the range 1–2 kHz Data from one of Moore's (1973) subjects show a deviation from this form at frequencies above 4–5 kHz, which he interpreted as evidence for a change from a temporal analysis at the lower frequencies to a place mechanism at the higher frequencies Nordmark argued that most evidence from frequency discrimination studies supports the theory of a temporal analysis as the main mechanism for discrimination Our data do not contradict this theory Temporal analysis may well be the common physiological function for detecting a frequency difference as well as a frequency sweep Differences in detection sensitivity might be explained by different ways in which the auditory system utilizes the temporal properties of the signal The place theory alone seems an unlikely explanation of the influence which the duration of the ramp had upon thresholds in the present study

Intensity

Previous studies using tone pulse pairs (Harris, 1952, Konig 1957) as well as continuous FM (Zwicker, 1952, Maiwald, 1967) have shown essentially the same influence of sound intensity on frequency discrimination as the present study, namely increasing thresholds with decreasing sound level below 40 dB HL and rather constant threshold above this level at least up to 80 dB Harris (1966), using tone pulse pairs at 45 dB SL in a background of white noise, found a similar relationship when plotting thresholds as a function of signal-to-noise (*S/N*) level The similarity indicates that *S/N* level is the important variable whether determined by an external masker or by the internal noise level of the auditory system

SUMMARY AND CONCLUSION

1 Discrimination of frequency ramps of a continuous pure tone was studied in ten young subjects with normal hearing
2 The mean threshold was found to be approximately 3 Hz at 1 kHz base frequency and 60 dB HL for ramp durations below 200 msec At longer ramp durations (>200 msec) the threshold was about 5 Hz
The absolute value of the threshold for frequency change was found to be approximately constant for low frequencies up to 1 kHz above which it increased
4 Thresholds increased with decreasing signal level at 1 kHz below 40 dB HL Above this level thresholds remained constant at least up to 80 dB HL
5 No significant difference was found between thresholds for positive and negative ramps
6 Intra individual variation was smaller than inter individual variation
7 It is concluded that for short ramp durations (<200 msec) discrimination depends on the difference between base frequency and plateau frequency For longer ramp durations (>200 msec) discrimination is based on detection of the actual frequency sweep

ZUSAMMENFASSUNG

Die normale Frequenzunterschiedsschwellen sind untersucht worden wobei lineare Frequenzrampen eines Dauertons als Reiz benutzt worden Fur kurze Rampendauer (<200 mSek) hat die Wahrnehmung seinen Grund im Unterschied zwischen Ruhefrequenz und Plateaufrequenz Die durchschnittliche Schwelle war da ungefähr 3 Hz für 1 kHz Ruhefrequenz Fur langere Rampendauer (>200 mSek) beruht die Wahrnehmung auf der Entdeckung der eigentlicher gleitender Frequenz Zunehmende und abnehmende Frequenz in den Rampen gab dasselbe Resultat Die Frequenzunterschiedsschwelle war für Ruhefrequenzen bis zu 1 kHz beinahe konstant (2–3 Hz) Bei höhere Ruhefrequenzen vergrösste sie sich und war bei 4 kHz etwa 14 Hz

Ein Tonpegel von 40 bis 80 dB HL hat keine Einwirkung auf die Unterschiedsschwellen aber 20 dB HL verursachte eine signifikant höhere Schwelle als 40 db HL Die intra individuelle Variation der Unterschiedsschwellen war kleiner als die inter individuelle Variation

Die aktuelle Ergebnisse werden im Verhaltnis zu fruheren Frequenzdiskriminationsdaten diskutiert

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OTOSCLEROSIS SURGERY

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Abstract Results of otosclerosis surgery using the

on fascia has become the preferred method because of its stability and relative lack of inner ear complications Inner ear damage is mostly due to perilymph fistulae which should be promptly recognized and repaired since revision after the ear has become deaf does not restore hearing Some late inner ear losses may be due to reactions to foreign material introduced with the graft Bilateral operations should not be done if the first operation was difficult or caused vertigo for several days

Since Shea (1956) made the first successful stapes footplate removals and sound-conducting system reconstruction for otosclerosis, the basic operative principle has remained the same These operations have been associated with occasional mishaps, which in the course of time have been more fully understood and some of them discussed in detail in recent articles (Dawes et al 1973, Burtner & Goodman, 1974, Smyth et al 1975) It seems that while the benefits are fully recognized a cautious approach is gaining ground as the sometimes very grave but admittedly few late sequelae of surgery are coming to light

We have now analysed our combined material operated on in the Department of Otolaryngology, University of Oulu from 1964 The patients were followed up postoperatively at least once a year, their subjective symptoms were recorded ears inspected and hearing tests made During this period, several techniques have been used for sound conduction reconstruction This makes it possible to compare the effectiveness of these techniques and the risk of the complications involved

MATERIAL AND METHODS

During the period 1964 to 1973 primary surgery for otosclerosis was performed in 482 ears (385 patients) The following survey consists of 456 ears (360 patients), in the case of 26 ears the postoperative follow up was less than 2 years which had been fixed as the shortest observation period Fifty patients (13 8%) had unilateral and 310 bilateral hearing impairment Of the latter group, 203 cases (65 5%) had operations on one ear, and of the remaining 107 cases, 96 had bilateral operations generally with a one year interval Of the remaining 11 bilateral cases, 8 had undergone stapes surgery in one ear elsewhere and 3 had a radical operation on one ear because of a chronic middle ear infection

The operative method consisted in removal of all or part of the footplate The reconstruction was effected with either a polythene tube House stainless steel wire, or the posterior or anterior crus The window was sealed with temporal muscle fascia in all cases using a polythene tube Fascia was also used in the great majority in the other reconstruction

Table I *Stapes reconstruction and bilaterality in 456 ears*

Type of reconstruction	Unilateral hearing impairment	Bilateral hearing impairment		
		Unilateral operation	Bilateral operation	Total operated ears
Polythene tube	17	80	76	173
Steel wire	9	49	65	123
Posterior crus	22	55	54	131
Anterior crus	1	6	5	12
Mobilization	1	13	3	17
Total ears	50	203	203	456

groups but, in altogether 16 of these ears, the wire or crus was placed on top of a piece of gelfoam In a small number of ears mobiliza tion of the footplate only was carried out Table I shows the distribution of patients into various subgroups according to surgical method

The hearing was evaluated by pure tone and speech audiometry (pure tone audiometer calibrated to ISO-standards) Masking was employed according to the principles described earlier using insert receivers (Palva & Palva, 1962) and keeping the masking level unchanged at pre- and postoperative bone conduction measurements

RESULTS

The overall results of the primary surgery are shown in Table II The highest figures for permanently good results are found in the groups in which the patient's own crura were used for reconstruction, and the poorest in cases subjected to mobilization only After primary surgery 3 ears became poorer in hearing, all in the group of stainless steel wire Taking together the groups involving footplate removal (439 ears), overall primary success was obtained in 95 4% and 83 2% retained this gain over the total observation period

Permanently Improved Ears

The average improvement for 500, 1 000 and 2 000 Hz during the observation period is shown in Fig 1 The one-year results obtained with polythene tubing were the best, significantly better than with posterior crus ($p<0\ 005$) At five years the results with the tube were equal to those with posterior crus, the curve for the latter was remarkably stable while both tubing and wire showed a downward sloping trend with the passage of time

The 6-months hearing results in the 373 pri-

Table II *Results of stapes surgery in 456 ears*

Type of reconstruction	Hearing improvement: Permanently improved		Late drop to pre-operative level		Total		No change in hearing (±10 dB)	Worse after surgery (>10 dB)	Total
	Ears	%	Ears	%	Ears	%			
Polythene tube	147	85 0	23	13 2	170	98 2	3		173
Steel wire	92	74 8	18	14 6	110	89 4	8	3	123
Posterior crus	119	90 8	18	8 3	129	98 5	2		131
Anterior crus	11	91 8	1	8 3	12	100 0			12
Mobilization	4	23 5	8	47 1	12	70 6	5		17
Total	373	81 8	68	13 2	433	95 0	18	3	456

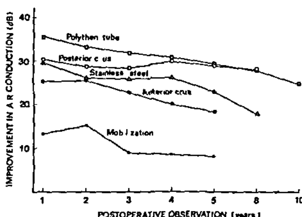


Fig 1 Air conduction hearing gain related to years of observation the reconstruction method as a parameter

mary operations are shown in Table III The ears with non measurable bone conduction values at some frequency (Table V) and the ears showing no improvement after surgery (Table II) are excluded On the average, the hearing levels for the three middle frequencies in cases with footplate removal were preoperatively between 53–57 dB whereas the average level in ears treated by mobilization was significantly lower, 44 3 dB ($p<0\ 05$) According to follow up averages, the best air conduction values, for polythene tube and posterior crus, were around 27 dB All groups with footplate removal were highly significantly ($p<0\ 001$) better postoperatively, the mobilization group as a whole did not, however, differ significantly from the preoperative value The bone conduction average values were significantly better postoperatively in the first three groups ($p<0\ 01$) while in the last two groups the difference, owing to the small number of cases, was not statistically significant

Table IV compares the hearing improvement in terms of air–bone gap If the preoperative bone conduction was taken as reference level, then the gap nearly closed in the two best groups (tube and posterior crus) The gap was largest in cases treated by mobilization, as much as 23 1 dB Comparison of air–bone gap and postoperative bone conduction levels reveals distinctly larger values, between 11 5 to 15 2 dB in the four best groups, which also indicates a distinct improvement in postoperative bone conduction averages

Ears with Severe Hearing Loss

In all groups of reconstruction there were ears which are not included in Tables III and IV because bone conduction was so low as to make recording impossible at 2000 Hz, and in some cases even at 1000 Hz The outcome as regards air conduction is summarized in Table V The final air conduction, though not reaching the useful level, showed distinct improvement in speech threshold, which made the use of a hearing aid easier

Revision Operations

Altogether 56 (12 3%) ears were later revised because of suspected fistula or because of depressed hearing without vertigo, in 8 cases two or more operations were made in the same ear bringing the total up to 68 revision operations The operative findings are listed in Table VI

Table III *Air and bone conduction values in 373 primary operations (ISO hearing level)*

Type of reconstruction	Air conduction				Bone conduction			
	Preop		Postop		Preop		Postop	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Polythene tube	56 9	16 7	27 7	14 1	24 8	12 2	16 3	11 6
Steel wire	57 0	15 3	32 4	13 4	23 6	12 1	19 6	12 7
Posterior crus	53 1	13 8	26 7	11 6	22 0	11 0	16 7	10 3
Anterior crus	55 4	15 5	33 4	11 9	22 0	11 9	18 1	12 1
Mobilization	44 3	10 8	38 6	12 7	16 4	3 8	15 7	10 4

Table IV *Air–bone gap in 369 primary operations*

Type of reconstruction	Preoperative		Relative to preoperative BC		Relative to postoperative BC	
	Mean	S D	Mean	S D	Mean	S D
Polythene tube	32 1	10 0	3 1	10 8	11 5	8 2
Steel wire	33 4	10 4	8 8	11 3	13 5	8 2
Posterior crus	31 2	9 4	4 5	11 6	10 1	7 4
Anterior crus	33 4	11 9	12 1	8 5	15 2	6 9
Mobilization	27 8	8 1	23 1	12 5	21 4	11 1

It appears that except for mobilizations, the percentages for revision in the various reconstruction groups are of the same order of magnitude, varying on each side of 10% Of the single causes, perilymph fistula occurred most frequently in connection with polythene tube (4 ears), and necrosis of the long process of the incus (4 ears) was found only in this group The most frequent cause of hearing loss using wire was poor contact in the window (8 ears), a too short wire having shifted from the centre towards the window margins or even on to the promontorial bone rim Using posterior crus, the most frequent cause was development of adhesions from the crus to the promontorial window margin (6 ears) with consequent limitation in movement In a total of 4 ears the crus had become short and atrophic and in 3 it had moved out of the window

Hearing after Revision

1 *Polythene tube*

In three ears with no primary improvement, revision surgery also failed but hearing remained at the preoperative level Of 16 ears with primary improvement and good bone conduction, 5 had complications which will be discussed later In 11 ears the average post-revision hearing was no better than before revision

2 *Stainless steel wire*

Revision surgery was made in 14 ears In 8 non complicated cases revision surgery was successful, improving the prerevision air conduction average from 57 9 dB to 33 8 dB

3 *Posterior crus*

In this group there were 16 uncomplicated ears In 2 cases with malleus or incus fixation (Table V) good hearing was obtained by direct columellization from the window to the drum using a transposed incus In the remaining 14 ears the average postrevision air conduction after replacement of the crus with a steel wire was 31 8 dB, the prerevision figure having been 39 6 dB (preoperative 46 9 dB)

Table V *Results in ears with severe hearing loss*

Type of reconstruction	Preoperative air conduction (dB)		Postoperative air conduction (dB)		Number of ears
	Mean	S D	Mean	S D	
Polythene tube	82 9	18 9	60 1	19 7	8
Steel wire	94 2	8 4	72.0	10 4	9
Posterior crus	73	–	45	–	1
Anterior crus	–	–	–	–	–
Mobilization	85	–	72	–	2

Table VI *Revision surgery in 56 ears (68 revisions)*

Operative findings	Polythene tube (*N*=173)	Stainless steel (*N*=123)	Posterior crus (*N*=131)	Anterior crus (*N*=12)	Mobiliza tion (*N*=17)	Total (*N*=456)
Perilymph fistula	4	2	1			7
Suspected fistula not found	1		1			2
Malleus head fixation			1			1
Incus fixation			1			1
Loss of incus long process	4					4
Atrophy of stapes crus			3			3
Poor contact in window	4	8	3	1		16
Poor contact with incus	2	1				3
Adhesive process	1	1	6			8
Refixation of footplate					8	8
No reason found		2				2
Total	16 (8 7%)	14 (11 4%)	16 (12 2%)	1 (8 3%)	8 (47 0%)	56 (12 3%)

4 Anterior crus

The single revision in this small group, in which the shortened crus was replaced by a polythene tube, resulted in a permanent gain from 52 to 32 dB

5 Mobilization

In one of the 17 ears some glue secretion was found in the middle ear at the primary opera tion and, as simple mobilization was success ful, nothing further was done In 9 other ears the fixation was so limited that initial pressure on the stapes mobilized it immediately and the procedure was terminated In 7 ears after removal of the stapes superstructure a float ing footplate developed and its removal was thought too risky However columellization was made from the incus with wire or tube

The prerevision average air conduction threshold (50 dB) improved to 33 8 dB in the 7 non complicated ears of the total 8 revision ears In all these ears the footplate was re moved and either tube, wire, or crus used for new sound conduction

Complications

Various types of complications occurred in 19 patients (4 2%) and they are listed in relation to the various operative procedures in Table VII The immediate complications consisted of one infected and two dry drum perforations at the posterior quadrant The infection sub sided rapidly with antibiotics and all three per forations healed under a cigarette paper patch The serious immediate complications, viz 3 fistulae, were all recognized and promptly

Table VII *Complications in 456 operated ears*

Type of reconstruction	Immediate postoperative complication: Drum perforation, Infected	Drum perforation, Dry	Fistula	Late postoperative complication: Infected drum perforation	Fistula	Deaf ears
Polythene tube (*N*=173)			1	2	3	2
Stainless steel (*N*=123)			2			1
Posterior crus (*N*=131)		2			1	2
Anterior crus (*N*=12)	1					
Mobilization (*N*=17)						1
Total 456	1 (0 2%)	2 (0 4%)	3 (0 6%)	2 (0 4%)	4 (0 6%)	6 (1 2%)

reoperated on the second or third postoperative day as tinnitus, vertigo, spontaneous nystagmus and complete loss of hearing had appeared The prostheses were found to be dislocated and were readjusted In all three cases the final outcome was satisfactory hearing

The late complications included two infected perforations, one of which needed myringoplastic repair after healing In neither case was inner ear function at risk Late fistulae were found in four ears In three of these the polythene tube had perforated the fascia and the tip was in the vestibulum, the symptoms appearing 6 months, 3 years and 8 years after operation, respectively The first patient's hearing improved after revision to the preoperative level but the other two ears became deaf, one of these after mild meningitis The fourth patient had also mild meningitis and became deaf 5 months postoperatively and a fistula was found in the upper window margin while the crus was well in place After revision there seemed to be some hearing initially but observation at 3 months showed the ear to be deaf

In addition to the 3 deaf ears associated with the late fistulae, 3 more ears became deaf during the follow up time (total 1 2%) In one of these, the reconstruction was made with polythene and in another with posterior crus on gelfoam The third case of deaf ear occurred after revision of a previously mobilized ear, reconstruction being done with a wire on fascia In none of these was an apparent cause found at the revision operation

DISCUSSION

At present time otosclerosis surgery is quite well standardized as far as one of the factors is concerned viz *removal of the footplate partly* or wholly Mobilization is no longer considered adequate and the very moderate gain to be obtained and the likelihood of refixation following mobilization are amply demonstrated even by our few cases One group remains, the floating footplate—half of our cases—where indications still may exist for a conservative approach, saving footplate removal until refixation has occurred Persistent *attempts to remove a floating footplate may* lead to its loss into the vestibule with subsequent damage to membraneous structures

Various materials are being widely used to cover the oval window and there are numerous methods of reconstruction from the incus We have been extremely hesitant to cover the oval window with only absorbable gelatin sponge as seven of our 16 patients had difficulties postoperatively In fact a recent report deals with deleterious effects upon the cochlea of gelatin sponge, which contains formaldehyde (Shenoi et al , 1975) We do not think that the use of vein, perichondrial or fascial grafts as such makes any difference and we have used fascia mainly because of the easy access to this tissue through our short endaural incision However, any material resulting in a thin membrane should not be used with prostheses that are not firmly anchored to the incus or which have a bevelled end not becoming incorporated into the tissues (polythene tube)

Our follow-up data with the three methods of reconstruction, viz polythene tube, posterior crus and steel wire, show that the best results were reached with the first two We increasingly favor the posterior crus, advocated e g by Hough (1975) and Goodhill (1974), since it is the patient's own tissue and guarantees the best possible contact with the incus We now employ steel wire only if the posterior crus is fractured above the level of the foot plate and becomes too short

In the case of standard stapedectomies, the primary operation generally leads to lasting hearing improvement in about 85% of the cases The number of revisions in any particular series depends much upon the anatomic state of the window, which may be very unfavorable (narrow niche, thick footplate extending deep into the vestibule) Persistent efforts may have been made after primary failure to obtain a gain Based on our experi-

ence, these cases should preferably be left alone, and a hearing aid be recommended, since too persistent a revision is likely to lead to cochlear damage The primary operation offers the best chance of obtaining a good hearing gain The gain after revision surgery is always poorer, in many cases there is no gain and hearing in some ears may become worse In our series polythene tube in the window seemed to have a particular tendency to cause marked tissue fibrosis and thus reduce both the safety and success of revision

Almost the only early serious complications of stapes surgery are perilymph fistulae following a dislocated prosthesis and graft If these mishaps are promptly recognized and the ears revised, no permanent damage need occur Late fistulae, on the other hand, carry a *different prognosis If repaired at the stage* when the hearing fluctuates, the result is generally good, but if the ear has become deaf, the hearing will not return Repair of the fistula should, nevertheless, be made to relieve the patient of vertigo

Even if granulomatous reactions have been almost non existent (one ear) in our series there is every chance that the two ears deaf without apparent cause may be due to some foreign material introduced with the graft It is surprising how easily anything less than the most meticulous handling of the fascia can cause incorporation of starch particles from *the end points of instruments touched by the* glove or of small tissue threads from the surgical covers into the fascia We realized the latter possibility many years ago by seeing these green coloured threads in the fascia already placed in the window They also proved nearly impossible to remove without discarding the whole graft In a few cases this foreign material may have faced the vestibule and thus remained unnoticed and may be a cause of some unexplained late cochlear damage in many series Our method for guarding against contamination of the fascia is to take it with clean nontouched instruments, place it immediately *between two ampicillin soaked* gelatin sponge surfaces, and uncover it only when picking it up for insertion into the window An illustrative case of another, similar foreign tissue reaction has been reported relating to the use of Ringer solution (Burtner & Goodman, 1974)

We have not used the piston technique at all except in difficult obliteration windows, since we fear that some of the plastic may later become toxic to the cochlear fluids Indeed something to this effect was recently reported by Dawes et al (1973), whose scanning electron microscopic pictures gave evidence of a rather rough microscopic surface of teflon piston tips

The question of bilateral operations in our series made in 107 patients (34%) is important Recently Smyth et al (1975) discussed this *matter and came to the conclusion that only* unilateral operations should be made This is supported by our figure of 1 2% deaf ears, and it should be noted that late mishaps may occur many years following a successful primary outcome Though in this series we have abandoned this policy on many occasions, we now advise against bilateral operations in all cases with advanced footplate otosclerosis In our experience the situation generally is similar on both sides and the risk of late damage may be considerable On the other hand, if one ear has been restored to nearly its normal state using half footplate re*moval and posterior crus on the fascia* we still operate the other ear after one year if the patient so wishes

One good rule in otosclerosis surgery is to keep the suction tip out of the window once the footplate has been perforated The vestibule should always be full of perilymph and, provided this is the case, the patients do not have postoperative vertigo Even in the most experienced hands this is not possible in absolutely every one of the cases Excessive sucking of perilymph may endanger the integrity of the membranous structures, and in such cases we advise against operating the other ear

ZUSAMMENFASSUNG

Die Resultate der Otosklerosenchirurgie mit Fußplatten entfernung und verschiedenen Methoden der Steigbugel rekonstruktion werden in 456 Ohren (360 Patienten) besprochen Primar erfolgreiche Resultate fand man in 96% der Fälle und 83% bewahrten ihren Gehorgewinn wahrend der ganzen Observationsperiode Das Crus posterior uber der Faszie ist wegen seiner Stabilitat und des *relativen Mangels der Komplikationen die* favori sierte Methode geworden Die Beschadigung des inneren Ohres beruht meistens auf der Entstehung eines Perilymphenfistels, welches sofort festgestellt und revidiert werden sollte, da man das Gehor nach einer Ertaubung nicht wieder herstellen kann Einige spate Ertaubungen konnen wegen fremder Materialien, die mit der Faszie inkorporiert worden sind, verursacht sein Bilaterale Operationen sollte man nicht durchführen wenn die erste Operation technisch schwer gewesen ist oder dem Pa tienten wahrend mehrerer Tage Schwindel verursacht hat

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NON-EXPERIMENTAL AURAL PATHOLOGY IN SOME PROSIMII

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(Received November, 7, 1975)

Abstract Pathological findings in the hearing organ of three Prosimii are described followed by remarks on anatomical dispositions of significance in experimental surgery

After tabulating pathological manifestations found in the hearing organs of three species (*Saimiri sciureus* rhesus and baboon) it promised to be of interest to investigate disease *found in the ear of Prosimii*, at the base of primate development and exempt of experimental measures Treeshrew (Tupaia), Slow ris (Nycticebus), and the Lesser Galago were explored in sectional series of the entire cranial base Material was most obligingly released by Dr Ernest A Peterson Chief of Auditory Research Department of Otolaryngology, University of Miami Florida Prepared by the celloidin technique, the specimens were sectioned in the horizontal plane and stained with hematoxylin eosin Albeit only a single series of each above-named *species was available, the comparative rarity* of the material made this study desirable It was completed by microphotography, executed by Mr Lloyd Matlovsky

Information on the aural pathology of the ear as found in monkeys in their natural habitat, before experimental intervention or even before prolonged stay in a competent animal farm or laboratory, is conspicuously lacking (Kelemen, in Benirschke, K *Pathology of Laboratory Animals*, J Springer, New York, in press)

FINDINGS

(1) *Treeshrew* (Tupaia)

Numerous pneumatic spaces of the middle ear contained serous secretions in the free central space, in the oval and the round window, around the promontory and around the facial nerve In the hypotympanum the secretion took on a denser character Circumscribed patches of suppuration surrounded the incudo-malleolar articulation In the inner ear, serous and sero-fibrinous secretions filled the basal turn of the cochlea Serous effusions were present around *the twin cristae and in the inferior* semicircular canal Around the pharyngeal tubal orifice the mucous membranes were engorged, next to the orifice the vascular conglomerate (Kelemen, 1955) was well distinguished

The signs enumerated above were partly restricted to the ear of one side

(2) *Slow Loris* (Nycticebus coucang)

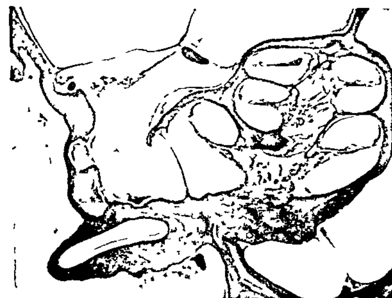
The tympanic membrane was retracted The pneumatic spaces of the middle ear showed serous and hemorrhageous content Serous secretions blocked the tubal corner They embedded the *incudo* malleolar articulation,



Fig 1 Lesser Galago Cavernous protuberance from the wall of the external auditory canal blocking the view to and through the tympanic membrane

behind a large tympanic perforation Secretions filled the niche of the round window and were present around the promontory They filled the bulla and buried the stapes and the facial nerve The canal of the latter showed a dehiscence of the canal wall at the anterior aspect, against the lateral crus of the stapes, with prolapse of the nerve At one side a stapedial artery was distinguishable The cochlea showed the strong bony arm between the cochlear apex and the wall of the bulla, the basal turn was filled by secretions which filled the posterior canal The vestibular aqueduct, around its middle course, carried a conspicuous extravasation

As in Nycticebus, the ear on one side showed the above enumerated changes in a much higher degree than on the other



Figs 2–5 Slow Loris
Fig 2 Middle ear from promontory to hypotympanum filled by serous secretions The hardly prominent subiculum makes the niche of the round window very shallow The tympanic membrane is retracted The facial canal wall shows a dehiscence of its entire tympanic wall The cochlea is free of infection



Fig 3 In the middle ear the ossicles are embedded in predominantly catarrhal secretions with some spots of suppuration The basal turn of the cochlea is filled by secretions the remaining turns are free with well preserved Corti organs The internal meatus shows some serous content Posterior crista and cupula are intact Some hypotympanic cells are filled by massive suppuration A stapedial artery is clearly visible at one side

(3) *Lesser Galago* (Galago senegalensis)
The external canal wall showed the characteristic protrusions Serous accumulations filled the middle ear, epi- and hypotympanum, besides several pneumatic spaces Around the promontory serous masses had accumulated, mixed with blood Serous content was seen in he vestibule, the cochlea remained free ile these were the findings on one side, the other contained only a minimal amount of serous secretions

ANATOMICAL REMARKS

Napier & Napier (1967) stated that in all prosimian families the floor of the middle ear is ballooned out to form a tympanic bulla In Treeshrew, for the bulla, Van Valen (1965)



Fig 4 The ossicles are embedded in serous masses The facial nerve is exposed without a canal wall to the inflammatory secretions Massive suppuration accumulated in the hypotympanum The cochlea remained free, with the vestibular walls carrying a thin cover of secretions



Fig 5 The incudo-malleolar articulation is surrounded by muco-purulent masses The thick engorged lining of the external meatus is seen representing besides the mural protrusions another obstacle to visualize the tympanic membrane from the outer meatus

assumed entotympanic origin, consequently it cannot be considered a homologue of the primate bulla Werner (1960) in Tupaiideae found the bulla to be large He cleared the conditions around the tympanicum (membrana tympani and its ring) Outside the tympanic ring, which forms the lateral wall of the auditory bulla, the lumen of the external canal is much narrowed, if not practically closed by protrusions from the walls (Fig 1) Accessibility to the middle and inner ear from the external canal is not possible Meyer (1931) had already emphasized this when, even with specially constructed ear specula, he was unable to survey the tympanic membrane (in baboon) in a satisfactory way This obstacle for surgical work is amply compensated for by the "huge" bulla in Tarsioidea (Hill, 1955), being made up of a single spacious pneumatic cell open against the medial wall of the cochlea In all the forms here discussed the bulla was already present, as a considerable protrusion The bony septum described by Beatty (1927), and mentioned by Hill (1956) and Tumarkin (1957), separating the antero-medial from the postero-lateral compartment of the bulla, was well developed This bony septum connects the cochlear apex to the wall of the bulla (Figs 2 and 3) While in lower forms, e g rodentia, the cochlea with vestibulum is practically freely suspended amid the pneumatic lacework, here a fixation with the strong bony bridge becomes definite The pneumatic cellwork in general is here characterized by large and consecutively fewer units

While in Rhesus (Kelemen, 1968) a subiculum, concealing the view from the direction of the tympanic membrane to the round window, is lacking and thus facilitates considerably the access to the inner ear, here the walls bordering the niche of the round window presented a very diversified profile With the external canal already effectively closed for surgical approach, this disposition of the subiculum has only minor significance To penetrate the inner ear through the round window, amputation of the external ear will be necessary

Experimental surgery on Prosimii can be expected to expand and to lend significance to the conditions here sketched

A stapedial artery was seen in Slow Loris Werner described its presence in Prosimiae (*sic*), inclusive of Tupaiideae

PATHOLOGY

Two of the three items showed serious middle ear invasion, the inner ear was less involved In all cases pathology was restricted mainly to one ear with slight participation of the other Any search for nonexperimental pathology should be influenced by the factor that disease which may have been present at arrival, might disappear during the stay in animal farms or laboratories On the other hand diseases that develop later may turn out to be different from those present at the time of acquisition (Lapin & Yakovleva, 1963) The comparative rarity of tympanic perforations as seen in higher monkeys (Kelemen, 1968) can be explained by more effectual drainage through the short and straight eustachian tube All pictures show a *retracted tympanic membrane* Whether this funnelshaped profile is normal in these animals could not be learned from the extant anatomical descriptions

Another reminder of conditions in higher monkeys is the evidence that middle ear inflammation rarely penetrated the capsule of the inner ear Nor did any penetration in the direction of the endocranium appear Besides anatomically efficient boundaries it seemed to represent a tendency of "toning down' of the virulence of infection from middle to inner ear

Further tabulation of pathological manifestations must await the availability of additional material

ZUSAMMENFASSUNG

Pathologische Veranderungen im Hororgan von drei Prosimii werden beschrieben nebst anatomischen Bemerkungen in bezug auf experimentelle Chirurgie

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HEMIFACIAL SPASM

Nerve Block with Phenol under Electromyographic Control

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Abstract Seven cases of hemifacial spasm are described Six were operated upon with intra-osseal exposure of the facial nerve In all 6 cases transient peripheral facial paresis developed When the paresis disappeared the patients had a recurrence of the spasms As an alternative to surgical procedures a method for selective nerve block under E M G control with 3% phenol is described and 5 cases have been treated with this method Ambulatory treatment with deposition of 3% phenol in the main branch of the facial nerve appears to have an equally good effect to the surgical methods hitherto used Examination with E M G suggested that the hemifacial spasm is of central origin

Hemifacial spasm (i e frequent shock like contractions of the facial muscles) is relatively uncommon, but when it occurs it is often felt to be a serious social handicap by the patient It is easily distinguished from other kinds of involuntary facial contractions such as synkinetic and dyskinetic movements, epilepsia partialis continua and myokymia (leading article, *British Medical Journal* 1975) by its typical clinical picture (leading article *British Medical Journal* 1972) and electromyographic pattern (Bumm et al 1972, Hjort & Willison 1973)

In hemifacial spasm the muscle convulsions or twitches consist as a rule of episodes of tonic–clonic contractions of the muscles innervated by the facial nerve The musculus orbicularis oculi is generally affected first the twitchings subsequently spreading to the muscles of the upper lip and finally to all those on one side of the face The stapedial muscle may also be affected (Diamant et al , 1967) The condition is thus usually confined to only one half of the face although bilateral cases have been described (McCabe, 1970) The spasm is sometimes mild and in such cases spontaneous remissions have been observed In others the attacks may assume a more progressive character The contractions occur irregularly and are usually not associated with pain When pain occurs it is relayed via the trigeminal nerve and has a neuralgiform character The spasms may be triggered and the intensity increased by more or less natural facial movements, such as chewing or laughing (Lathrop, 1953, Benos, 1972, Potter, 1972, Pulec, 1972) but also by light (Cawthorne, 1965) and by emotional stress (Ehni & Woltman, 1945, Williams et al , 1952, Potter, 1972, Pulec, 1972) The spasms even occur during sleep (Greenwood, 1946, Potter, 1972)

Symptomatic hemifacial spasm has been described as being caused by aneurysm of the basilar artery, neoplasm in the cerebellopontine angle, arachnoiditis of the posterior fossa (Cushing 1916, Campbell & Keedy, 1947, Laine & Nayrac, 1948, Kramer & Eckman, 1972, Janetta 1972) tumour or local infection close to the facial nerve (Cawthorne, 1965) The condition may also be seen in patients in

whom recovery from Bell s palsy is not complete Usually, however, the cause of the condition remains unknown (idiopathic hemi facial spasm)

Etiologically, two different hypotheses have been put forward, one implying that the spasms are elicited centrally either because of an irritation or a loss of inhibition at a nuclear or supranuclear level (Wartenberg, 1952, Cawthorne, 1965, Eckman et al , 1971, Benos, 1972, Bumm et al , 1972), the other, that the spasms are caused peripherally by a state of chronic irritation such as a fibrous constriction of the nerve sheath or oedema of the facial nerve somewhere along its course (Harris & Wright, 1932 Williams et al , 1952, Proud, 1953, Esslen, 1957, Zulch, 1970, Fisch & Esslen, 1972, Pulec, 1972, Sadé, 1974) There is also electromyographic evidence suggesting a central etiology (Bumm et al , 1972) as well as a peripheral (Fisch, 1957)

No specific therapy has been devised Medical as well as surgical measures have been tried with varying results during recent decades Two types of operative technique may be distinguished, the first one aiming at a decompression of the facial nerve and a decrease the pressure exerted upon it in the Fallopian canal, the second aiming at diminishing the number of axons in the nerve in order to reduce the impulses from reaching the facial muscles Both techniques but especially the latter imply a balance between an elimination of the spasms and the creation of facial palsy Simple decompression of the vertical portion of the nerve sometimes combined with a graded trauma has been performed by Williams et al (1952) Proud (1953) Cawthorne (1956) and with a more modern technique by Pulec (1972) who extended the decompression of the nerve from the stylomastoid foramen up to the internal auditory meatus Those who favour the second type of operative technique either transect the facial nerve trunk totally (Ehni & Woltman 1945 Gilliatt & Taylor, 1959) or partially (Scoville 1965, Lewis 1965, Celis Blaubach & Castillo 1974) or transect a varying number of the peripheral facial branches totally (Coleman 1937, Lathrop, 1953, Potter, 1972, Fisch & Esslen, 1972) or partially (German 1942, Greenwood, 1946, Wanke, 1947, Miehlke 1959, Diamant et al 1967, Sade, 1974) Most of these operations create more or less pronounced facial paresis which, when it regresses, usually is accompanied by a recurrence of the hemifacial spasm

Owing to the high frequency of recurrences after surgical procedures and the negligible effect of medical treatment non operative methods using physical or chemical nerve block of the main trunk or peripheral branches of the facial nerve have been devised (Schlosser, 1907, Greenwood, 1946, Bettag et al , 1961, Cawthorne, 1965, Totsuka et al , 1972, Wakasugi 1972) Phenol as a chemical blocking agent has been shown to have many advantages It was used by Khalili & Betts (1967) in a 2-3% solution in distilled water and had a mean duration of effectiveness of 308 days when used on 126 peripheral nerves Using these recommended concentrations of phenol we have blocked the facial nerve trunk peripheral to the stylomastoid foramen in cases of hemifacial spasm after having located the nerve trunk by electric stimulation The blocking procedure was followed electromyographically which permitted a fractionated blocking and thereby regulation of the intensity of the treatment

The purpose of the present investigation was to elucidate the pathophysiology of hemifacial spasm and to test the suitability of this simple non operative method using injection of phenol into the main trunk of the facial nerve

METHOD

The diagnosis of hemifacial spasm was made on the basis of clinical findings the course of the condition and EMG examination of muscles innervated by the facial nerve

Table I

No	Patient	Year of birth	Sex	Onset of spasms	Side affected	Pain	Facial decompression (month/year)	Recurr after (months)	Sensori neural hearing impairm	First inj of phenol (month/year)	Recurr after inj	Effect on the spasm	Duration of effect (months)
1	K M	1914	♂	1968	R	–	10/70	12	–	7/73	2	Good	4–24
2	H L	1914	♂	1968	R	–	8/71	12	+	–	–	–	–
3	K Å	1922	♂	1968	R	+	11/71	10	–	–	–	–	–
4	S J	1914	♀	1969	R	+	1/72	12	–	4/73	1	Good	0 5–12
5	H H	1914	♂	1969	R	+	9/71 and 3/73	15 resp 6	–	3/73	2	Good	3–20
6	M S	1916	♀	1962	R	–	7/72	10	+	12/73	0	Good	21
7	KG A	1917	♂	1974	R	–	No op	–	–	10/74	0	Good	11

EMG guided injection

As a rule, the musculus frontalis, musculus orbicularis oculi musculus orbicularis oris and the platysma were examined and the spontaneous as well as the voluntary muscle activity was recorded with concentric needle electrodes The facial nerve was stimulated transcutaneously and the conduction time on both the healthy and the diseased side was determined With the aid of an injection needle insulated except at its tip, the trunk of the facial nerve was located behind the mandibular branch by determination of the point where the smallest stimulus impulse produced an EMG response in the muscle innervated by the facial nerve Thereafter 1–2 ml of a 3% phenol solution was injected through the needle into the nerve and the effect was simultaneously recorded electromyographically The injection was continued until a facial paresis was just recorded

MATERIAL

The material (Table I) consisted of 7 patients (5 males and 2 females) aged 53 to 61 years The hemifacial spasm had existed for 6 months up to 11 years Most patients reported that external stimuli such as heat, cold, and mechanical pressure in the innervated area could cause spasms In 3 of the patients the spasms were painful Six of the patients were previously operated upon with intratemporal facial nerve exposure and decompression All 6 had a recurrence of the spasms Four of these patients accepted further treatment using nerve block with phenol The first injections were made 15–33 months after the operation One patient was primarily treated with nerve block by phenol (case 7, Table I)

RESULTS

Operative treatment

Of those 6 patients operated upon, all had a more or less pronounced transient postoperative facial paresis The paretic condition lasted between 6 months and one year The hemifacial spasm recurred 6–15 months after the operation though then less frequently and less severe than before Two patients developed mild sensorineural hearing impairment postoperatively on the side operated upon (Table I) Otherwise, no operative complications occurred

Phenol treatment

The effect on the spasms was good in all 5 cases, i e the patients became symptom free Three of the patients had recurrences (Table I), but the spasms disappeared each time the injection was repeated The effect of the injections lasted up to 24 months (Table I) and did not become shorter from one injection to

whom recovery from Bell's palsy is not complete Usually, however, the cause of the condition remains unknown (idiopathic hemifacial spasm)

Etiologically, two different hypotheses have been put forward, one implying that the spasms are elicited centrally either because of an irritation or a loss of inhibition at a nuclear or supranuclear level (Wartenberg, 1952, Cawthorne, 1965, Eckman et al , 1971, Benos, 1972, Bumm et al , 1972), the other, that the spasms are caused peripherally by a state of chronic irritation, such as a fibrous constriction of the nerve sheath or oedema of the facial nerve somewhere along its course (Harris & Wright, 1932, Williams et al , 1952, Proud, 1953, Esslen, 1957, Zulch, 1970, Fisch & Esslen, 1972, Pulec, 1972, Sade, 1974) There is also electromyographic evidence suggesting a central etiology (Bumm et al , 1972) as well as a peripheral (Fisch, 1957)

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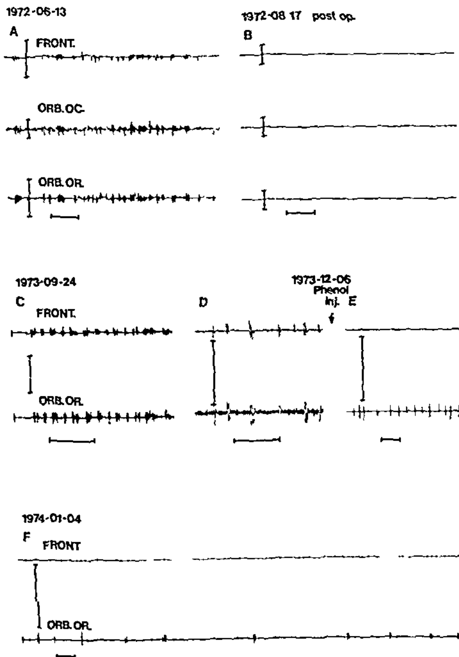


Fig 2 Patient M S E M G recorded with concentric electrodes from musculature innervated by facial nerve on right side (A) Preop (B) 6 weeks after op (exposure of facial nerve) (C) 14 months after op recurrence (D) 17 months after op Inj of phenol (E) Paresis of upper branches (F) Mild persisting twitches of orbicular muscle of the eye persisted and were still demonstrable after 1 month Calibration 1 mV, 1 s

Case 6 (M S 57 years)

This patient had had right-sided hemifacial spasm for 10 years, without pain but with considerable inconvenience Intra-osseal exposure of the facial nerve with slitting of the nerve sheath was made in July 1972 The operation had a good effect but the symptoms recurred within one year Phenol block the

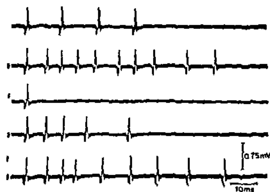


Fig 3 Patient KG A E M G recorded with 5 ms delay—line from orbicular muscle of the eye The first motor unit in each twitch started the sweep Each sweep represents a discharge Observe the high frequency with which the motor units discharge (75–150 Hz) Cal bra tion 0 75 mV 10 ms

controlled the spasms However, EMG still showed small rhythmic discharges though these failed to produce any visible contrac tions (Fig 2)

Case 7 (KG A 57 years)

In April 1974 this patient had right sided hemi facial spasm in the area innervated by the two branches of the facial nerve Treatment 0 4 g Tegretol (later 0 6 g) daily for some months had no effect The musculus orbi cularis oculi on the right side showed rhythmic discharges of motor unit potentials The dis charges were rhythmic with frequencies up to 100 to 150/sec These spastic twitches oc curred in sequences of 2–20 units per time (Fig 3) After injection of 2 ml 3% phenol solution into the trunk of the facial nerve subtotal paresis appeared in the area in nervated by the facial nerve After injection the spontaneous twitches and the spontaneous EMG discharges disappeared The course during the first 3 months after injection was uneventful apart from a moderate paresis When seen again 4 months after the injection the patient no longer had spastic twitches but he still had mild paresis of the lower branch of the facial nerve corresponding to the mus culus orbicularis oris

DISCUSSION

The 7 cases of idiopathic spasm described showed typical clinical and EMG findings No patient had had a facial nerve palsy before the spasms and no predisposing factors could be found In all patients the symptoms were severe and in 3 cases were associated with pain Pain is usually quite a rare symptom, but when it occurs it is neuralgiform and very troublesome The pain may be relieved by blocking the trigeminal nerve but this affects neither the intensity nor the frequency of the spasm (Diamant et al , 1967) An interesting association between trigeminal neuralgia and hemifacial spasm has been proposed by Harris & Wright as early as in 1932 Histologically similar changes in the trigeminal and facial nerve ascribable to the respective condition have been described by Kumagami (1974) However, definite proof connecting the two diseases is still lacking EMG failed to reveal any evidence of a peripheral origin of the spasms The rhythmic discharges occurred roughly simultaneously, but not quite syn chronously in the muscles involved At each discharge the individual motor units were ac tivated separately with frequencies as high as 150 Hz, which is much higher than those seen on maximum voluntary contraction On elec tric stimulation of a facial branch only mus cles innervated by this branch were activated Abnormal synapses between the various facial branches could therefore not explain why muscles innervated by different branches were activated roughly simultaneously *A central nervous system origin of the condition there fore appears most likely* This question will be more closely discussed in a forthcoming article (Elmqvist et al , 1977)

Medical treatment of hemifacial spasms with analgetic, sedative, antidepressant and antiepileptic drugs have with few exceptions (Benos, 1972, Crabtree, 1972) proved unre warding (McCabe, 1970, Sadé, 1974) Surgery has usually been followed by recurrence within one or two years although many pa tients may report some improvement This is

also our experience After intra osseal exposure of the facial nerve and slitting of the nerve sheath all 6 patients became symptom free but developed temporary facial paresis Later on, however, when the spasms recurred the intensity was somewhat less but the patients were still incapacitated

In 2 of the operated cases, slight sensori neural impairment of the hearing developed (Table I) probably due to vibrations or noise exposure from the operating drill

The usually discouraging results of surgical treatment has stimulated the search for alternative methods Injection treatment was tried already in 1907 by Schlosser, who used absolute alcohol, and later on by Bettag et al (1961) Cawthorne* (1965) and Wakasugi (1972) Procaine (Cawthorne, 1965) and hydrocortisone (Esslen 1957) injected around the facial nerve trunk has also been advocated *but without definite success* Extensive experimental work by Khalili & Betts (1967) has shown that phenol as a 2–3% solution in distilled water is a splendid agent for blocking the impulses in nerve fibres Based on their positive experiences we have applied this method to patients with hemifacial spasm In all, we have treated 5 patients who have been observed for 3 years or more Three of them were blocked more than once, and each time the effect upon the spasm was good, i e the spasms disappeared As after surgical procedures the spasms recurred after the injections, in our cases at most after 24 months However the combination with guiding simultaneous EMG has been a therapeutical advance as the nerve trunk can be localized exactly thereby permitting an injection of phenol not larger than just sufficient to produce relief of the spasms It is a further advantage that the procedure may be repeated many times without any difficulty and is easily performed at an ambulatorium

Among our 7 patients there were 5 men In the literature there is a slight female predominance but the material presented here is small and no statistical conclusions can be drawn regarding the sex ratio An interesting point, however, is that all the 7 patients had their spasm on the right side Could it be that hemifacial spasm is more common on the dominant side in right handed persons, especially since there is some evidence of a central origin of the spasm? There is no evidence of this in the literature and the fact that all our patients were spastic on the right side could be a mere coincidence

ZUSAMMENFASSUNG

Beschreibung von sieben Fällen mit hemifazialem Spasmus Sechs wurden mit intraossealer Freilegung des Gesichtsnerven operiert In samtlichen sechs Fällen kam es zu einer vorubergehenden peripheren Fazialislahmung Nach Verschwinden der Lahmung erfolgte ein Wiederauftreten der Spasmen EMG Befunde deuten auf eine zentrale Genese der Spasmen Als eine Alternative zur chirurgischen Behandlung wird eine selektive Blockierung des Nerven unter EMG Kontrolle beschrieben Ambulante Behandlung mit Deponierung von dreiprozentigem Phenol im Hauptstamm des N fazialis erscheint ebenso effektiv wie die bisher gebrauchlichen chirurgischen Maßnahmen

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THE EFFECT OF INTRACISTERNALLY INJECTED OUABAIN ON PERILYMPH ELECTROLYTE CONCENTRATIONS

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Abstract A means for altering the ionic composition of perilymph utilizing the cochlear aqueduct and cerebrospinal fluid in chinchillas is introduced A decrease in sodium and an increase in potassium concentration in perilymph was observed after ouabain was injected into cerebrospinal fluid The time course of changes in the perilymph paralleled the CSF change but with a time lag which had not reached equilibrium within 60 minutes

Although cationic ratios in the inner ear fluids seem to play a major role in the transduction of vibratory energy, their origin and the mechanisms for maintaining fluid homeostasis are not yet clearly understood

Many investigators hold the opinion that perilymph is an ultrafiltrate of blood derived from capillaries in the spiral ligament (Schreiner 1966, Silverstein, 1966) Cerebrospinal fluid (CSF) was also implicated in perilymph formation of several species by the demonstration of a patent cochlear aqueduct connecting the subarachnoid space and the scala tympani (Gisselson 1949)

Sodium and potassium concentration gradients which exist between the intracellular and extracellular spaces are dependent on an active transport system which exchanges sodium and potassium ions across cell membranes It has been suggested that the Na-K-ATPase system in the cochlear tissues is responsible for maintenance of the cation gradients between endolymph and perilymph or blood (Kuijpers, 1969)

Ouabain, a cardiac glycoside, is known to inhibit the adenosine triphosphate (ATP) dependent sodium–potassium pump (Schatzmann, 1953, Hajdu & Leonard, 1959, Skou, 1965) It has been shown that cochlear microphonic potentials could be inhibited by ouabain (Chou, 1970, Konishi & Mendelsohn, 1970, Sellick & Johnstone, 1974) Konishi & Mendelsohn (1970) also reported that, when ouabain in Ringer-Locke's solution was perfused through the perilymphatic space, a marked depression of cochlear potentials occurred in conjunction with an increase of sodium and a fall in potassium levels in the endolymph

Unavoidable disturbances in the inner ear fluid homeostasis are probably caused by perfusing the cochlea with drug or artificial solutions prior to electrophysiological studies as has been in the past The purpose of the present study was to determine first, whether intracisternally injected ouabain could cause comparable perilymph electrolyte changes without destruction of the cochlear integrity and second, to examine the extent to which

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Table I *Effect of intracisternally injected ouabain on the sodium and potassium concentrations (mEq/l) in CSF and perilymph of chinchillas*

	Serum		CSF		Perilymph	
	Na	K	Na	K	Na	K
0	158 8±0 5[a] (18)[b]	4 3±0 1 (18)	168 0±0 5 (19)	3 0±0 1 (19)	171 3±1 7 (15)	4 7±0 2 (15)
5 min	155 4±1 2 (8)	4 2±0 3 (8)	148 6±3 2 (5)	9 5±1 0 (5)	170 4±0 7 (7)	5 3±0 6 (7)
15 min	155 8±0 9 (15)	4 7±0 3 (5)	149 2±1 9 (4)	10 0±0 7 (4)	163 3±1 6 (5)	9 8±1 3 (5)
30 min	155 0±0 3 (3)	4 6±0 1 (3)	152 3±1 4 (6)	16 6±0 9 (6)	165 7±1 4 (5)	12 1±1 3 (5)
60 min	156 7±2 7 (6)	6 7±1 0 (5)	148 9±0 7 (4)	23 8±1 8 (4)	163 2±0 6 (5)	15 1±1 0 (5)

[a] Standard error of the mean
[b] The numbers in parentheses indicate the numbers of samples

changes in CSF ion concentrations influence the chemical composition of perilymph

MATERIALS AND METHODS

Forty healthy chinchillas were used in this study After anesthesia with urethane, a tracheostomy was performed and ouabain (10^{-3} M, 0 2 cc/kg), was injected into the subarachnoid space The same volume of CSF was withdrawn prior to the ouabain injection to prevent the formation of a pressure differential between the subarachnoid and perilymphatic spaces As soon as breathing became labored, the animals were intubated and placed on a respirator until sampling was completed At various time intervals (0, 5, 15, 30, and 60 minutes) after ouabain injection, samples of blood, CSF and perilymph were collected Blood was drawn from the jugular vein and CSF was collected from the subarachnoid space by inserting a needle through the atlanto occipital membrane Perilymph samples were taken by inserting a thin glass tube drawn to a fine point through the round window membrane

Sodium and potassium were determined simultaneously using a flame photometer (Instrumentation Laboratory model 143) calibrated with a standard solution of 140 mEq/l of sodium and 5 mEq/l of potassium Determinations were made using 5 μl of all samples taken and diluted to 1 ml with a diluent containing 15 mEq/l of lithium

RESULTS

The data obtained from this study are presented in Table I and Fig 1 The sodium concentration in CSF dropped about 11% during the first 5 min post-injection and remained at or near that value for all successive time periods studied The potassium concentration in CSF appeared to increase continuously over the time periods studied In perilymph,

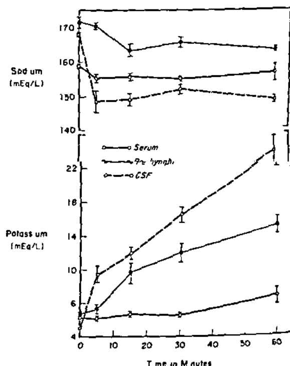


Fig 1 Effect of intracisternal ouabain injection on the sodium and potassium concentration of perilymph CSF and serum of the chinchilla

sodium concentration had not fallen significantly in 5 min but dropped about 4 7% over the remaining time periods The potassium concentration in perilymph also rose steadily over the intervals of these studies A time lag was observed between perilymph and CSF potassium increase for each experimental period No significant changes were observed in serum electrolyte concentrations

DISCUSSION

In an earlier study, we demonstrated the passage of intracisternally injected substances (urea and albumin) into perilymph (Juhn & Guzowski, 1973), by a slow diffusion through the cochlear aqueduct The rate of increase in perilymph concentration of injected substances in relation to that of CSF was some what dependent on the molecular weight of the test substances

Ouabain is a strong inhibitor of Na–K–ATPase which is responsible for the maintenance of cation gradients across cell mem branes Ouabain is known to cause the influx of sodium into cerebral cortex slices and the efflux of potassium brain cells by inhibiting Na–K–ATPase system (Yoshida et al 1961, Bonting et al 1962, Quastel 1970) The rapid decrease of sodium and increase of potassium in CSF after intracisternal ouabain injection clearly demonstrates a similar effect of this drug on the brain tissues which are bathed by SCF The changes in ionic composition of perilymph observed in this study can be attributed both to an indirect effect of ouabain mediated by the change in composition of CSF and to a direct effect of ouabain on tissues lining the perilymphatic space Iinuma (1967) reported the existence of Na–K–ATPase in the cochlear tissues Ouabain could inhibit the active ion transport system with resultant alterations of ionic concentration in the cochlear compartment The proportion of ionic changes in perilymph due to the involvement of tissues surrounding the perilymphatic tissue is not known Our results show the summation of changes caused by direct effects of ouabain on the cochlear tissues and by changes in ionic composition of CSF which subsequently influence the cochlear space

The results of the present study clearly reveal another means for changing the ionic composition of perilymph by altering the composition of CSF This is accomplished utilizing physiological routes without damaging the anatomical integrity of cochlear tissues Further studies are underway on the functional changes in the cochlea when ionic changes in perilymph are induced by this method

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ZUSAMMENFASSUNG

Die Beeinflussung der ionischen Zusammensetzung der Perilymphe uber dem cochlearen Aquädukt und die Zerebrospinalflussigkeit an Chinchillas wurde besprochen und eine Methode mitgeteilt Ein Abfall von Natrium und ein Ansteigen des Kaliumspiegels erfolgt nach Injektion von Strophanthin in die Zerebrospinalflussigkeit Der Zeitverlauf der Ionenveränderungen in der Perilymphe erfolgte nach eingänglicher Verzogerung gleichsinnig mit Veranderungen im zerebralen Liquor aber nach einer Stunde war noch kein Gleichgewicht eingetreten

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MIDDLE EAR PRESSURE DURING AND AFTER PROLONGED NASOTRACHEAL AND/OR NASOGASTRIC INTUBATION

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Abstract. Changes in middle ear pressure during and after prolonged nasotracheal and/or nasogastric intubation were studied in 47 patients who had been intubated for various reasons during 1–24 days. All the patients had a negative middle ear pressure, in 84% of the ears the pressure was −200 mm of water or less. In most ears the pressure fell rapidly after the intubation being most negative before extubation and during the first 2 days after. In all patients who could be followed the pressure returned to normal. The normalization was slow depending upon the duration of intubation. Possible causes, such as abolished ventilation of the rhinopharynx, insufficient swallowing, mechanical occlusion of the tubal orifice, and the influence of anaesthetic gases are discussed. During the period after extubation the most probable cause is irritative and inflammatory reaction of the mucosa leading to internal tubal occlusion.

Out of 4 children in whom post-mortem examination of the middle ear and Eustachian tube showed evident histopathological signs of incipient secretory otitis, 3 had a history of nasogastric intubation during the first month of life (Tos & Bak-Pedersen, 1976). In another autopsy series, 7 children and prematures exhibited histological changes in the middle-ear mucosa. These changes consisted in vascular dilatation and proliferation, round-cell infiltration, metaplasia to stratified epithelium, increased goblet cell density and new formation of mucous glands (Tos & Bak-Pedersen, 1976). Four had a history of nasotracheal or nasogastric intubation. Similar changes were found in 5 adults, 3 of whom had had nasotracheal or nasogastric intubation during the last few days and 2 during the last 3 months before death. The histological changes were interpreted as sequelae to tubal occlusion caused by the intubation.

Consequently, it was felt justified to study the ventilation of the middle ear during and after prolonged intubation. It is quite conceivable that its ventilation gets reduced as a result of reduced ventilation of the rhinopharynx and insufficient swallowing during the intubation. From clinical practice, it is well known that during prolonged intubation patients occasionally complain of a sensation of fullness in the ears, but the literature does not appear to contain concrete data on changes in the middle ear pressure during and after prolonged intubation.

MATERIAL AND METHODS

The material comprises 47 patients, 16 females and 31 males, who had been treated with prolonged nasotracheal and/or nasogastric intubation in the Intensive Care Unit during the period September to November 1975. Only one was a child, aged one year, the remainder adults. Nine in the age range 31–50, 21 from 51 to 60, 8 from 61 to 70, and 8 from 71 to 90. Thirty underwent planned operation—on the heart (15), lungs (4), peripheral vessels (5), or abdomen (6). The intubation from the operation was extended to the post-

Table I *Number of ears with middle ear pressure at −200 mm H_2O or less during and after intubation Number of ears which never reached this level*

	Intubation		Total	
	Uni lateral	Bi lateral	*n*	%
During	9	9	18	19
After	5	6	11	12
During and after	11	39	50	53
Never	5	10	15	16
Total	30	64	94	100

operative period The unoperated group comprises 17 patients with acute respiratory failure due to cardio pulmonary basic disease (7), rib fracture (1), poisoning (1), subarachnoid haemorrhage (1), gastroenterological (5) or nephro urological diseases (2) Nasotracheal intubation was by polyethylene tube (Portex), nasogastric intubation by a plastic tube

On the day of intubation the patients had otological examination, and the middle ear pressure was measured by tympanometry using the Madsen ZO 70 impedance apparatus, frequency 220 Hz The tympanometry was repeated, in both ears, at suitable intervals, initially every or every other day, later every three or four days until the pressure returned to normal, the patient died, or was discharged No patient had manifest acute or chronic diseases of the upper respiratory tract At least the operated patients had been thoroughly examined preoperatively but without tympanometry

Table II *Number of ears with middle ear pressure at first tympanometry of −200 mm H_2O or less (fast fall) and of more than −200 mm H_2O (gradual fall)*

	Intubation		Total	
Fall	Uni lateral	Bi lateral	*n*	%
Fast	16	42	58	62
Gradual	14	22	36	38

Table III *Further fall of middle ear pressure in 36 ears which at first tympanometry had a pressure of more than −200 mm H_2O*

	Intubation		Total	
Further fall	Uni lateral	Bi lateral	*n*	%
To −200 or less	9	12	21	58
To more than −200	3	3	6	17
No further fall	2	7	9	25
Total	14	22	36	100

RESULTS

On the basis of several hundreds of tympanometric measurements at different junctures on 94 ears, the findings may be summed up as follows

1 All 47 patients had negative middle ear pressure of varying degree during and after the intubation This applied to 93 ears, but not to one—despite unilateral intubation of almost one day's duration

2 A middle ear pressure of −200 mm of water or less was found on at least one occasion in 84% of the ears, either during or after the intubation, but most often (in 53%) both during and after (Table I) Only 16% had a pressure of more than −200 mm but as a rule it was between −100 and −175 mm

3 62% of the ears showed a rapid fall of pressure which was already at the first tympanometry, usually on the day after intubation, −200 or less (Table II) In 31% of these ears there was a further fall subsequently In 38% the pressure altered gradually after being at the first tympanometry more than −200 mm of water (Table II) In 58% of these ears (Table III) the pressure fell further, being at subsequent tympanometric measurement −200 or less In 17% it also fell, but never beyond −200 of water In the remaining 25% there was no further fall (Table III)

4 In principle, there were no differences in the pressure variations on unilateral intubation—nasotracheal in 12 patients and nasogastric in three—and bilateral intubation with

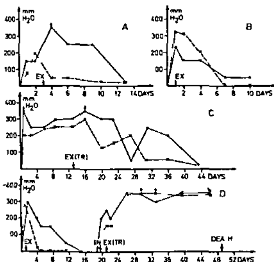


Fig 1 Examples of changes in middle ear pressure in 4 patients with unilateral intubation —— Intubated side , non intubated side *EX* time of extubation *TR* tracheotomy *IN* re intubation (A) Most negative pressure on the intubated (B) on the non intubated side (C) slow normalization after tracheotomy (D) rapid normalization of the pressure after the primary intubation After re intubation for 24 hours and tracheotomy a pronounced fall of middle ear pressure with a flat curve (arrow upwards) until death

a nasotracheal tube on one side and nasogastric on the other (Tables I–III) Among the patients having unilateral intubation there was a negative pressure on the contralateral side in all ears but one A rapid fall in pressure was found in the same number of ears (8) on the intubated as on the non-intubated side (Table II) In several patients, however, the pressure was on the whole more negative on the intubated side (Fig 1 A and C) and it was slower in returning to normal (Fig 1 C and D) than on the non intubated side In a few cases this was the other way about (Fig 1 B)

5 In bilaterally intubated patients there were no significant differences in the course and extent of the changes or in the period of normalization between the side of the fairly thick nasotracheal tube and that of the thin nasogastric tube (Fig 2)

6 There was a definite association between the degree of the negative pressure and the duration of intubation (Table IV) During the first 2 days of the intubation 62% of the ears, and during the third or fourth day 84%, had a middle ear pressure of −200 mm of water and less Among patients intubated for longer than 4 days the pressure was negative in practically all ears throughout the period of intubation—which in some cases was up to 24 days Prior to extubation, as a rule immediately before, the pressure was most negative, the majority of ears showing a flat tympanometric curve and more than three quarters a pressure of less than −200 mm of water (Table IV)

7 After extubation the pressure in most ears remained greatly negative for a day or two (Table IV), and in a few ears there was even a further fall (Table I, Figs 1 A and 2 D), and in three quarters of the ears the pressure was still −200 mm of water or less Three and four, and five and six days after extubation an increasing number of ears showed a pressure between −175 and 0, but more than half the ears still had a pressure of −200 mm of water or less Seven and eight, and nine and ten days after extubation more than one third of the ears had a pressure of −200 mm or less (Table IV) After the tenth day and up to 28 days after extubation a few ears still showed a more negative pressure There was a clear association between the duration of intubation and the

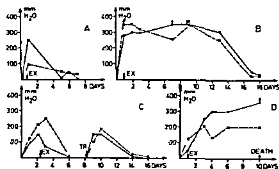


Fig 2 Examples of changes in middle ear pressure in 4 patients with bilateral intubation —— Nasotracheal nasogastric tube (A) Rapid (B) slow normalization after 24 hours intubation (C) normalization after intubation and again negative pressure after tracheotomy (D) slow fall in pressure especially after extubation and until death *EX* extubation *TR* tracheotomy arrow upwards flat tympanometric curve

Table IV *Middle ear pressure at various times during and after intubation*

Pressure	During intubation			Before extubation	After extubation				
	1st or 2nd day	3rd or 4th day	5th–24th day		1st–2nd day	3rd–4th day	5th–6th day	7th–8th day	9th–10th day
Flat curve	14	6	7	22	19	7	6	3	9
–300–325	18	5	4	16	18	14	2	2	1
–250–275	12	4	5	10	17	4	4	1	2
–200–225	14	21	2	19	10	10	6	4	1
–150–175	11	5	2	10	8	4	3	5	2
–100–125	15	2		3	3	9	5	2	7
–50–75	6	1		5	4	10	3	4	6
0–25	4			3	7	12	5	19	6
Total	94	44	20	88[a]	86	70	36	40	34

[a] Three patients died before extubation

degree of the negative pressure during the period after extubation

8 Normalization of the pressure to –25 and 0 mm of water occurred in all 31 patients (62 ears) (Table V) that could be followed As a rule, it was slow, with a gradual increase of pressure There was some association between the duration of intubation and the period of normalization, the pressure returning more quickly to normal in most patients intubated for a short time and more slowly in those intubated for a long time However, patients intubated for only 24 hours might show a negative pressure for up to 20 days after extubation (Fig 2B) Among patients who could not be followed because they died before (3) or after extubation (6) there had not yet been any signs of incipient normalization of the pressure Among those discharged to their homes (4) or transferred to other hospitals (3) the majority exhibited incipient normalization of the pressure

9 Normalization of the pressure was slowest in patients who had undergone tracheotomy in conjunction with nasotracheal extubation (Fig 1C) If, after a few days' extubation and normalization of the pressure, tracheotomy was done, the pressure fell again and was slow in returning to normal (Figs 1D and 2A) In these cases the absence of an air current through the rhinopharynx must have been responsible for a slower normalization than in non tracheotomized patients

10 At otological examination on the day after intubation the great majority of patients had a negative pressure, retracted drums, but no signs of effusion in the middle ear, also not in patients whose tympanometric curve was flat Paracentesis—done in the case of a patient who had had greatly negative pressure through several days—yielded no fluid Only a few patients complained of a sensation of fullness in the ears

Table V *Normalization of middle ear pressure after extubation related to duration of intubation expressed in numbers of ears*

Duration of intubation (days)	Days after extubation					Total
	2–5	6–10	11–15	16–20	25–30	
1	12	7	3	2		24
2	4	3	2	1		10
3	3	2	3			8
4	3		1			4
6		2	2			4
8		2				2
10		2			2	4
13			2		2	4
24			2			2
Total	22	18	15	3	4	62

DISCUSSION

To maintain normal ventilation of the middle ear, the rhinopharynx must be ventilated the Eustachian tube mechanically passable, and

its opening and closing mechanism adequately functioning There are several conceivable explanations of reduced middle ear ventilation during and after intubation

1 Abolished ventilation of and absence of air current through the rhinopharynx during the intubation period The rapid fall of pressure after the intubation, and especially the slow normalization of the pressure in tracheotomized patients indicate that ventilation of the rhinopharynx is of great importance However, it cannot explain the reduced ventilation of the middle ear after extubation After the patient has been extubated and the air current has been re-established, the middle ear pressure ought to return quite rapidly to normal, but it did not

2 Lacking or insufficient swallowing, which is indeed the most important factor in opening the Eustachian tube (Flisberg, 1966) The material did not include any patients with cranial trauma and long lasting unconsciousness True, the operated patients had had a blurred sensorium during the first postoperative day, but after that the great majority were fully conscious and cooperative Apart from the period under anaesthesia, swallowing was present in all patients, but it was insufficient while the tubes were *in situ* It may be imagined that during swallowing opening of the Eustachian tube is deficient in intubated patients, possibly because of an insufficient contraction of the musculus tensor veli palatini As the soft palate cannot shut off the rhinopharynx during swallowing, the condition may resemble that of cleft palate After extubation, however, swallowing was normal, and then the middle ear pressure ought to be rapidly normalized, but it was not

3 Mechanical occlusion of the tubal orifice may be imagined to be responsible in some cases for reduced ventilation of one side, but hardly in general, especially as unilateral intubation might cause changes in pressure in both ears Loch (1942) demonstrated tubal occlusion when placing a soft balloon in the rhinopharynx

4 Irritative and inflammatory reaction of the mucous membrane in the rhinopharynx and Eustachian tube can fully explain the persistent negative pressure and its further accentuation after extubation as well as its association with the duration of intubation, i e a greatly negative pressure and slow normalization in patients subjected to long lasting intubation The nasal and rhinopharyngeal mucosa will react to the foreign body by increased secretion and oedema Owing to the absence of an air current and impaired ciliary function, there is difficulty in transporting the secretion to the pharynx, and it will accumulate in the remaining space Mechanical injury to the epithelium and consequent crusting as well as reduction of muco-ciliary clearance will increase stagnation of the secretion and inflammation The inflammation must also comprise the pharyngeal part of the Eustachian tube whose mucosa is identical with that of the nose and whose seromucous glands also become hyperactive, leading to increased secretion in the tube (Tos, 1971) The secretion from the Eustachian tube cannot be transported to the rhinopharynx, accumulating in the Eustachian tube and causing in combination with the mucosal oedema internal tubal occlusion Although the ventilation of the rhinopharynx and swallowing after extubation have presumably been normalized, and the rhinopharyngeal secretion has been mechanically eliminated, internal tubal occlusion will prevent a rapid normalization of middle-ear ventilation High doses of antibiotics with which the great majority of patients, and especially the operated ones, have been treated, have no doubt prevented bacterial infection which would otherwise easily occur under such conditions

5 The horizontal position reduces passive transport of secretion and increases the accumulation of secretion in the rhinopharynx Besides, ventilation of the middle ear in the horizontal position is reduced because of hydrostatic pressure in the vessels of the Eustachian tube and mucosal thickening (Perl

man, 1939, Moore & Miller, 1951, Ingelstedt et al, 1967, Rundcrantz, 1969) However, the patients were not positioned horizontally during the postoperative intubation, but elevated at least 30° After extubation most of them were able to sit or were ambulant, whereby these factors were eliminated

6 Nitrous oxide, used in combination with halothane or morphine in the intubation anaesthesia of the operated patients, diffuses to the middle ear and increases the middle ear pressure to +300 mm of water in persons with normal as well as in persons with impaired tubal function This is a phenomenon observed daily in myringoplasty The pressure increases for up to 10 minutes after the discontinuation of nitruous ocide inhalation in order thereafter to fall in 45 minutes to 0 mm of water or to the patient's original pressure (Thomsen et al, 1965, Rasmussen, 1967)

Absorption of the gases of the respiratory air from the middle ear to surrounding tissues and diffusion the other way have been studied by Ingelstedt & Jonson (1967), Elner (1970, 1972), and Elner et al (1971) There is constant absorption of oxygen and nitrogen from a normal middle ear, causing negative middle ear pressure which in the presence of normal tubal function is constantly eliminated during swallowing Under anaesthesia there is a duplication of the oxygen percentage, and there ought to occur an increase in oxygen diffusion to the middle ear, causing an increased middle ear pressure, especially as swallowing is abolished Under anaesthesia with 0 5–2% halothane in oxygen, however, Rasmussen (1967) found very slight changes in middle ear pressure, in a positive or in a negative direction Similar findings were made by him under intravenous anaesthesia using barbiturates and pure oxygen During the postoperative period most patients have received a supplement of oxygen during as well as after the intubation

Objective examination of the tympanic membrane at the institution of intubation showed no signs of effusion, also not in ears having a flat curve at the first tympanometry (Table IV) Thus, there is no evidence of the formation of a transudate after short lasting tubal occlusion, as observed in experimental tubal occlusion However, paracentesis was not done systematically in our patients On paracentesis Berry et al (1975) found no effusion in 18% of children having a middle ear pressure of −200 mm of water or less Continued investigations of the middle ear in intubated patients, in whom the duration of tubal occlusion can be accurately determined, affords good chances of elucidating the histopathology of tubal occlusion in man Studies conducted so far on autopsy materials (Tos & Bak Pedersen, 1976) have shown that tubal occlusion induces changes of the lamina propria consisting in vascular dilatation and proliferation, lymphocytic infiltration, differentiation of epithelial cells with an increase in goblet cell density, which gradually leads to epithelial metaplasia and the formation of mucous glands and thus sets up a possibility of exudation and increased mucous secretion with accumulation of secretion However, these processes are considerably slower in adults than in children in whom prolonged nasotracheal intubation will more rapidly lead to chronic secretory otitis

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ZUSAMMENFASSUNG

Bei 47 Patienten die aus verschiedenen Ursachen 1 24 Tage intubiert waren wurden Änderungen des Mittelohrdruckes wahrend und nach der prolongierten nasotrachealen und/oder nasogastrischen Intubation gemessen Alle Patienten hatten einen negativen Mittelohrdruck bei 84% der Ohren war der Druck −200 mm Wasser oder weniger Bei den meisten Ohren fiel der Druck schnell nach der Intubation und war am niedrigsten vor und in den ersten zwei Tagen nach der Extubation Die Normalisierung war langsam und war von Dauer der Intubation abhängig Mögliche Ursachen der Druckänderungen sowie fehlende Ventilation des Nasopharynx mangelhaftes Schlucken mechanische Obstruktion des Tubenostium und der Einfluss der anäste

tischen Gassen wurde diskutiert Nach der Extubation ist eine irritative und entzündliche Reaktion der Schleimhaut, die zu einen internen Tubenverschluss führt die wahrscheinlichste Ursache des niedrigen Druckes

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PARAMETERS FOR ARGON LASER SURGERY OF THE LOWER HUMAN TURBINATES

In Vitro Experiments

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Abstract On 8 human lower turbinates in vitro experiments were performed using an argon laser with a power of 1–10 W, a beam diameter of 0 2 and 2 mm For clinical laser surgery of the turbinates 1–2 W is sufficient Drilling incision coagulation and vaporizing experiments were done on the tissue of the lower turbinates The results of this laser surgery are discussed and compared with present-day surgical methods such as conchotomy cutting of the posterior ends by slinging and electrocautery of the lower turbinates

The subject of this paper is the possibility of replacing conventional surgery of the lower turbinates, which is applied more particularly in vasomotor rhinitis, by a bloodless and more efficient method Present-day surgical therapy aims at reducing the tissue, which has strongly dilated vessels and edema, either through electrocautery of the turbinates, whereby the mucosa is largely spared If the mucosa is badly swollen, the tissue is removed surgically by conchotomy which is more radical and produces more blood, or the enlarged posterior ends of the lower turbinates are cut off by slinging

Laser surgery now offers the possibility of coagulating, vaporizing and burning the tissue This technique has been demonstrated in the ear, nose and throat by many authors (Andrews & Moss, 1974, Fruhmorgen et al , 1974, Goldman et al , 1968, Hobeika & Rockwell, 1973, 1972, Jako & Strong, 1973, Kaduk & Fruhmorgen, 1975, Lenz & Eichler, 1975, Lenz et al , 1976, Strong & Jako, 1972, Strong et al , 1974)

The application of the laser brings the following advantages It avoids loss of blood by sealing the capillaries, small veins and arteries Laser surgery permits bloodless removal of precisely determined amounts of tissue by vaporization of volumes in the region of μm^3 to cm^3 Transection of the tissue without bleeding is also possible (Mussiggang & Rother, 1974, Lenz et al , 1976) By the contactless mode of operation and the aseptic effect of the beam, laser surgery reduces the danger of infection The argon laser is probably more suitable than other laser types for the desolation and reduction of vessels because of its wavelength in the blue green which reacts selectively on the red shining vessel system (Lenz & Eichler, 1975, Lenz et al , 1976) In the following in vitro experiments the parameters for the argon laser, i e laser power and focusing conditions, are investigated for bloodless conchotomy, incision and vaporization of the posterior ends of the lower turbinates and laser cautery of the lower turbinates

Table I *Summary of the experiments Present-day surgery of the lower human turbinates is compared with possible laser surgery*

Type of experiment	Possible application	Present-day method
1 Drilling 2 Perforation	Laser cautery (punctiform)	Electrocautery
3 Incision 4 Vaporization	Laser surgery of the posterior ends	Mechanical slinging of the posterior ends
5 Coagulation	Laser cautery (strip form) with coagulation (and vaporization)	Conchotomy

MATERIALS AND METHODS

Eight lower turbinates from 6 deceased men (1–6 days post mortem, preserved at 1°C) 61–72 years of age were used An argon laser (Schafer & Seelig, 1970) with 1–80 W in multimode operation at a wavelength of 0 488 μm and 0 514 μm (blue-green) with almost equal power was used The beam of 16 mm diameter and a divergence angle of 10^{-3} was focused by a quartz lens with a focal length of 20 cm The diameter of the focal spot is 0 2 mm In some experiments (Series 4 and 5) a defocused beam with 2 mm diameter was used

Procedure

The turbinates were obtained by dissection They were fastened with a clamp at the upper end and fixed to a horizontal movable plate The mucosa of the medial area of the turbinate was adjusted parallel to the plate The laser beam was reflected vertically on to the medial mucosa of the turbinates by means of a mirror The beam was first focused by a lens onto the surface of the mucosa using very low power (approx 50 mW) The diameter of the spot was about 0 2 mm with a field depth of ±5 mm (giving an increase in beam diameter of 5%) Several series of experiments were carried out

Drilling The focus was adjusted to about 5 mm from the lower edge of the turbinates Drilling experiments at a laser power up to 10 W (power density 3×10^4 W/cm^2) were performed The bony area of the turbinates prevents perforation

Perforation About 1–2 mm from the lower edge of the turbinates 30 perforations were produced on a total of 5 turbinates at a power between 0 5 and 10 W The depths of the holes and the drilling times were measured The experiments were performed in the lower middle section of the turbinate, where the thickness varies but little

Incision Using the focused laser beam the posterior ends of the turbinates were cut off

Vaporization With a defocused beam, 2 mm in diameter, the posterior ends of the turbinates were vaporized and burned The power was 10 W at a power density of 300 W/cm^2

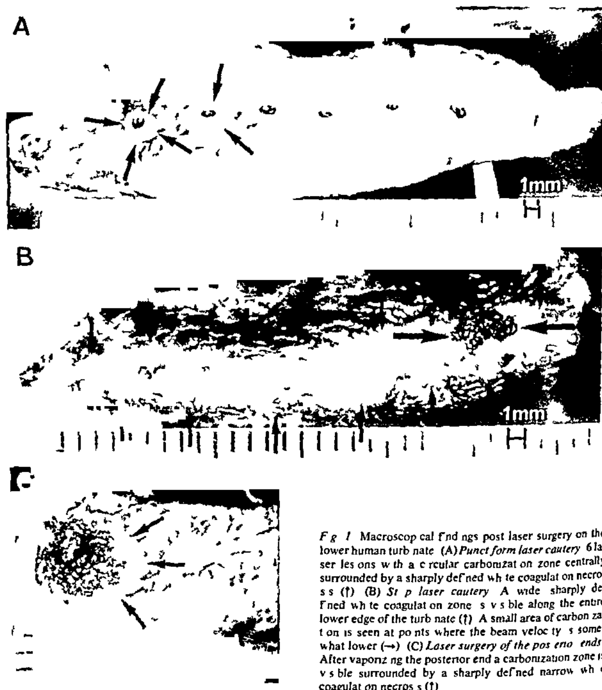
Coagulation The defocused laser beam, 2 mm in diameter, and 2–3 W power, was moved over the lower edge of the turbinates from the anterior to the posterior end The tissue of 2 turbinates was coagulated The geometry of the turbinates and the lesion and the radiation period were measured

All turbinates were fixed in 10% formaldehyde and decalcified with EDTA natrium The preparations were embedded in Paraplast Several sections were obtained from each specimen The slides were stained with hematoxylin and eosine and examined through a light microscope

RESULTS

The experiments are summarized in Table I

1 +2 *Drilling and perforation* Immediately after laser impact, quick vaporization of the tissue occurs with a circular carbonized zone surrounded by a sharply defined white coagulation necrosis (Fig 1A) No perforation of the bony region of the turbinates occurred within 2 min at powers of 1–2 W The turbinate was perforated in 1 min when the focus was adjusted to within about 5 mm of the lower edge The corresponding time at 10 W was



F g 1 Macroscop cal f nd ngs post laser surgery on the lower human turb nate (A) *Punct form laser cautery* 6 la ser les ons w th a c rcular carbonizat on zone centrally surrounded by a sharply def ned wh te coagulat on necro-s s (↑) (B) *St p laser cautery* A wide sharply de f ned wh te coagulat on zone s v s ble along the entire lower edge of the turb nate (↑) A small area of carbon za t on is seen at po nts where the beam veloc ty s some what lower (→) (C) *Laser surgery of the pos erio ends* After vaporiz ng the posterior end a carbonization zone is v s ble surrounded by a sharply def ned narrow wh e coagulat on necros s (↑)

several seconds The depth of the hole was 4–5 mm In the periphery area where no bone exists the perforation time is as shown in Fig 3 A for powers of 1–10 W The drill ng velocity is shown in Fig 3 B A power of 1 W was sufficient to produce a perforation

3 +4 *Incision and vapori ation* of the pos terior ends With a power of 10 W the focused beam cuts the posterior ends in 19 sec with an incision area of 6×5 mm² The burning and vaporizing period in respect of the posterior ends is longer than when cutting with laser A volume of about 100 mm³ is vaporized in 21 sec and 200 mm³ in 47 sec using a defocused beam Macroscopically a carbonized area is seen centrally surrounded by a sharply de



Fig 2 Light microscopical findings following laser surgery on the lower human turbinate (A) *Laser cautery* Cross-section of 3 adjacent drill canals (↑) of Fig 1A H E ×25 (B) Higher magnification of (A) showing a drill canal with a circular carbonization zone surrounded by a sharply defined narrow coagulation necrosis (↑) H E. ×250 (C) *Strip laser cautery* Cross-section of the coagulated lower edge of the turbinate of Fig 1B with carbonization and coagulation necrosis (↑) H E ×50 (D) *Laser surgery of the posterior ends* Longitudinal section of the carbonized posterior end of Fig 1C with a carbonization zone (1) in the centre of the lesion followed by coagulation necrosis (2) with a narrow zone of loose tissue caused by vaporization (3) H E ×180

fined narrow white coagulation necrosis (Fig 2C)

5 *Coagulation* of the lower edge: The defocused beam, 2 mm diameter, with 2–3 W power is able to coagulate the lower edge of the turbinates at a length of 35 mm in 70 sec (Fig. 1B). The width of the coagulated stria was 3–4 mm (Fig. 1B). The velocity of the

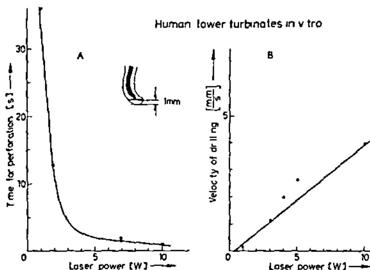


Fig 3 (A) Time required to perforate the lower turbinates of humans dependent on the laser power The position of the drilled hole is indicated (B) Drilling velocity calculated from (A)

beam movement was 0 5 mm/sec Some small black dots became visible at some points where the beam velocity was somewhat lower (Fig 1B)

Light microscopical findings

In experimental series 1 and 2 drill canals of nearly equal size were produced using the same laser power (Fig 2A) The drill canal shows a circular carbonization zone surrounded by a sharp bordered small coagulation necrosis (Fig 2B) In experimental series 3 and 4 a carbonization zone is visible in the centre of the laser lesion followed by coagulation necrosis with a narrow zone of loose tissue (Fig 2D) In series 5, a sharply defined coagulation necrosis is usually seen in the centre of the laser lesion (free margin of the lower turbinate) without carbonization Only in some areas is a central carbonization visible, surrounded by coagulation necrosis dependent on the applied laser dosis (Fig 2C)

DISCUSSION

The experiments were performed on non pathological turbinates of dead humans As a result the enlargement of the tissue due to increased blood supply and edema has to be taken into consideration However, the thermal parameter as heat conductivity of living and dead tissue is similar and heat transport due to the blood circulation is small during laser surgery (Eichler & Lenz, 1976) Thus the technical findings of the paper, such as laser power, drilling velocity, cutting, coagulation and vaporization seem to be fairly good approximate values for laser surgery of swollen turbinates with vasomotor rhinitis

The typical light microscopical findings on the lower human turbinates after laser surgery show a carbonization in the centre of the lesion, surrounded by a sharply defined coagulation necrosis Using a defocused beam with reduced power density only a coagulation necrosis could be established The effect of low power density laser radiation on the mucosa was studied in earlier papers (Lenz & Eichler 1975, Lenz et al , 1976)

An argon laser with powers smaller than 2 W is suitable for clinical laser cautery operations These lasers are commercially available in highly reliable versions Higher powers are dangerous due to the high drilling velocity Damaging of the medial wall of the maxillary cavity is possible after perforation of the turbinates If the laser cautery is performed in front of the bony region of the turbinate the operation is extremely safe

A laser incision of the posterior ends can be performed at 10 W in less than half a minute At about 2 W the time is estimated to be 5 times larger, i e 2–3 min Thus the same laser

which is suitable for cautery may be used for cutting the ends. Care must be taken to ensure that the surrounding tissue in the region of the main nasal cavity and of the epipharynx is not damaged by the beam.

For burning and vaporizing the posterior ends the operation period is longer, i e at 10 W about 1 min is necessary. If the tissue is enlarged by the blood supply the corresponding time becomes larger, proportional to the vaporized volume. Vaporizing the posterior ends of the lower turbinates using a defocused beam of 1–2 mm is much safer than laser cutting the posterior ends with a defocused beam The great advantage of both methods is the fact that no bleeding is to be expected

Laser coagulation or vaporization of the lower edges of the lower turbinates would appear to have the same advantage, i.e. no bleeding Using a defocused beam of about 2 mm in diameter the tissue can be reduced with a laser of 2 W in a controlled and rapid manner.

This paper shows that laser surgery of the lower turbinates is technically possible with an argon laser of about 2 W A rhinoscope with a facility for guiding the laser beam and an arrangement for drawing off the tissue vapour has to be constructed and manufactured for clinical use.

ZUSAMMENFASSUNG

An 8 unteren menschlichen Nasenmuscheln werden in vitro-Versuche mit einem Argon Laser mit Leistungen von 1–10 W, einem Strahlendurchmesser von 0 2–2 mm Diameter durchgeführt Es zeigt sich, daß für eine klinisch brauchbare Laserchirurgie an den unteren Nasenmuscheln Leistungen von 1–2 Watt ausreichen Es werden Versuche zum Durchbohren, Schneiden, Koagulieren und Wegdampfen des menschlichen Muschelgewebes durchgeführt Diese laserchirurgischen Ergebnisse werden mit den bisherigen chirurgischen Methoden der Conchotomie, des Abschlingens von hinteren unteren Muschelenden und der Muschelkaustik in Beziehung gestellt und diskutiert

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ONCOCYTIC CYSTS OF THE LARYNX

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Abstract Oncocytic cysts make up a pathologically well defined sub-group of cystic lesions in the larynx As a rule they originate from the ventricle and occur in middle aged or elderly persons Clinically these tumours are un characteristic Such oncocytic cysts are interpretable as retention cysts rather than actual new growths The question concerning their rare occurrence is discussed on the basis of 5 cases diagnosed within a period of 2 months

Oncocytes are epithelial cells having a granulated eosinophilic cytoplasm and small hyperchromatic nuclei They are common in salivary glands and in the glands of the respiratory-tract mucosa and their excretory ducts Furthermore, they have been demonstrated in other glandular tissue (Hamperl, 1937)

As a rule these cells are taken to represent a degenerative process They have been known under different names, e g Askanazy or Hürthle cells in the thyroid The epithelial component of Whartin's adenolymphoma in the parotid gland is composed of oncocytes Their cytoplasmic granulation is due to large mitochondria with longitudinal crests and an increased content of mitochondrial oxidative enzymes There is nothing to suggest that the oncocyte serves any special function in the organs where it has been found (Tremblay, 1969)

Oncocytic cysts of the larynx are considered rare The first case was reported by Notheri in 1946 Since that time 77 cases have been reported, as a rule isolated case reports or very small series

During the period September to November 1974 five oncocytic cysts of the larynx were diagnosed in the Pathology Department of the Copenhagen City Hospital We therefore felt prompted to draw attention to the clinical and morphological features of this disease which does not seem as uncommon as previously assumed

MATERIAL

The material comprises five histologically confirmed oncocytic laryngeal cysts removed surgically in the ENT Department of the Copenhagen City Hospital during the period September – November 1974

Case Reports

Case 1

A woman, aged 82, who had been suffering from chronic "laryngitis" for 30–40 years Three days before admission she felt pain on swallowing an ordinary meal of fish On physical examination the voice was found to be moderately pneumophonic, and laryngoscopy disclosed a red, glistening, pedunculated polyp measuring 1×2 mm on the left false cord, close to the anterior commissure and covered with normal-looking mucosa There were no signs of retained foreign body or mucosal injury The polyp was removed *in toto* by Kleinsasser microlaryngoscopy



Fig. 1. Oncocytic cyst showing papillary ingrowth into the luminal space. ×25.

Case 2

A woman aged 51 with a 3 week history of hoarseness and a tendency to cough. Physical examination revealed a roundish polypous mass 6×7 mm issuing from the left ventricle and covered with normal mucosa. The tumour was removed *in toto* by microlaryngoscopy.

Case 3

A woman aged 52 referred in July 1974 for chronic laryngitis. In the course of a protracted cold she had been hoarse for one month. Physical examination revealed marked prominence of the left false cord with a normal mucosal cover. There was also some thickening of the mucosa on the left true vocal cord. Biopsies from the prominent false cord and from the changes on the true cord showed mild non-specific inflammatory changes. However, as the symptoms persisted, microlaryngoscopy was performed 2 months later. This disclosed a 5–6 mm large roundish polypous mass of a bluish hue arising in the left ventricle and covered with normal mucosa. The polypous mass was removed *in toto*.

Case 4

A woman aged 57 referred because of a polyp on the right vocal cord. For 2 months the patient had been hoarse. Indirect laryngoscopy revealed swollen mucosa in the right ventricle partially covering the right vocal cord. The laryngeal mucosa was normal throughout without any discoloration. At microlaryngoscopy a cystic tumour of the ventricle was removed. It covered about the middle third of the right false cord.

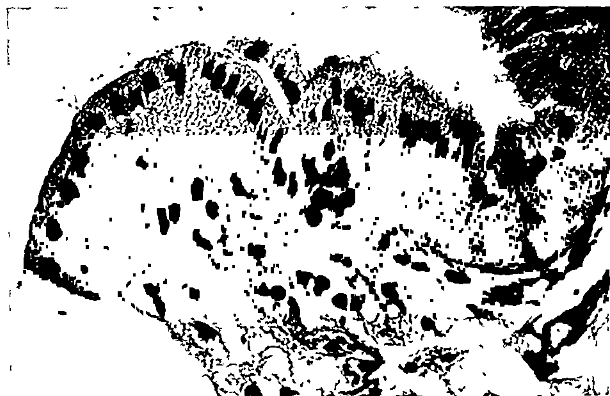


Fig 2 The characteristic epithelium of an oncocytic cyst ×400

Case 5

A woman, aged 60, referred for a neoplasm of the left vocal cord Three weeks previously she had developed hoarseness accompanied by irritative, non-productive cough Indirect laryngoscopy revealed thickening of the left false cord which covered the anterior two-thirds of the true cord The mucosa was slightly irregular, but showed no ulceration, coating, or discoloration Microlaryngoscopy revealed prolapse of the mucosa in the left ventricle, and it was removed piecemeal while cyst fluid oozed out

All the patients have been followed for 9–12 months after the operation, without exhibiting any signs of recurrence

HISTOLOGY

The cysts were uni or multilocular, of a diameter up to 1 cm They were embedded in loose connective tissue and always lay close to the surface, which was lined with respiratory epithelium

The cystic wall, lined with simple or pseudc stratified cuboidal or columnar epithelium was usually smooth, but in some areas ther were small papilliferous excrescences (Fig 1) In most sites the epithelium showed ample granular, highly eosinophilic cytoplasm, an the nuclei were usually pyknotic (Fig 2) A few typical goblet cells were observed, and in several places gradual transition from onco cytes to areas lined with columnar epitheliun like the normal epithelium in the glandula ducts

In the surrounding connective tissue ther were seromucous glands and excretory ducts often containing small areas of oncocytes

DISCUSSION

Laryngeal cysts may be divided into con genital and acquired which again may be sub

Table I *Reported cases of oncocytic cysts of the larynx*

	Author	Age	Male	Female	Nomenclature
1946	Nohteri	66	1		Cyst composed of oncocytes
1949	Som & Peimer	50		1	Oncocytic cystadenoma
1951	Heinz	80		1	Adenolymfoma
1956	Vosteen	83		1	Oncocytic adenoma
1956	Ash & Raum	58	1		Oncocytic cystadenoma
1960	Ellis	78		1	Adenoma
1961	Kuhn	65		1	Cystadenoma
1961	Heath	67		1	Eosinophilic granularcell cystadenoma
1961	Pinkerton & Beck	57–76	2	2	Eosinophilic granularcell cyst
1965	Møller & Ørntoft	59		1	Oncocytoma
1965	Capo	67	1		Oxyphilic adenoma
1966	Steiglich Petersen	51	1		Eosinophilic papillary cystadenoma
1966	Kleinsasser et al	64–74	2	1	Oncocytic adenoid hyperplasia
1967	Hubner et al	70		1	Oncocytic cyst
1967	Kroe et al	56–72		2	Oncocytic papillary cystadenoma
1967	Brandenburg	52		1	Oncocytic cyst
1968	Lennox et al	70		1	Adenolymfoma like tumours
1969	Ekedahl & Schnurer	59–66	2		Eosinophilic papillary cystadenoma
1969	Gallagher & Puzon	56–80	12	7	Oncocytic lesion
1970	DeSanto et al	Not stated		22*	Oxyphilic cyst
1972	Barton	64–72	1	1	Oxyphilic adenoma
1973	Mendonca & Ward	48–88	2	8	Oxyphilic adenoma

* Sex ratio not stated

divided into actual tumours or retention cysts Our oncocytic laryngeal cysts, as well as those reported previously, have occurred in middle aged or elderly persons, suggesting that they are acquired There have been reports of transition from oncocytes into columnar epithelium as found normally in glandular ducts, and ciliated oncocytes have also been described (Notheri, 1946) Moreover, neighbouring seromucous glands and excretory ducts often show focal areas with oncocytes This indicates that oncocytic cysts are retention cysts, formed by ectatic excretory ducts Accordingly, we felt that the term oncocytic cyst would be most apt There has been much disagreement concerning the nomenclature, as is apparent from Table I

Most oncocytic cysts of the larynx have affected the false cord or the ventricle, the remainder the subglottis, epiglottis, anterior commissure, arytenoid regions and true cord (Table II) The latter site is remarkable, as usually the true cord does not contain glands However, the cysts may have originated in adjacent excretory ducts

Grossly the oncocytic cysts of the larynx usually present as greyish red, soft tumours up to 1 cm in diameter, seldom larger Their cystic nature has rarely been recognized clinically

Histological appearances have invariably been analogous with our findings

The reported mean age of patients with oncocytic cysts of the larynx has been 64 years (48–88), as may be seen from Table I In our series the mean age was 60 (51–82)

Previous reports mentioning the sex have shown a slight female preponderance (55%), while all our patients were women However, the total number is too small to permit conclusions concerning a real sex difference In

Table II *Site of reported cases of oncocytic cysts of the larynx (own cases in parenthesis)*

Ventricle	43	(4)
False cord	19	(1)
Vocal cord	5	
Anterior commisure	4	
Subglottic	3	
Arytenoid	2	
Epiglottis	1	
Not stated	1	
Total	78	(5)

our series as well as in previous ones, the most outstanding sign has been hoarseness persisting for weeks or even years before the diagnosis is made. A few patients have also complained of pain (Møller & Ørntoft, 1965), stridor, or laryngeal obstruction (Notheri, 1946; Steglich-Petersen, 1966). Of those cases reported in the literature, 2 have been multiple (Gallagher & Puzon, 1969, Ekedahl & Schnurer, 1969), and 8 recurrent, but in these cases it is not quite certain that the primary operation was radical (Som & Peimer, 1949; Heath, 1961; Møller & Ørntoft, 1965; Ekedahl & Schnürer, 1969; Gallagher & Puzon, 1969) In all cases there has been normal mucosal cover, and clinically there has been no suspicion of malignancy

There have been no definite reports of malignant transformation of oncocytic cysts in the larynx Som & Peimer (1949) found oncocytes of ordinary appearance side by side with normal seromucous glands in the muscles of the vocal cord. It seems more likely that these were ectopic glands, rather than infiltrative growth, and indeed the patients were free of recurrence after 18 months.

Malignant transformation of actual tumours made up of oncocytes in other sites has been described in a few cases (Fayemi & Toker, 1974)

Cysts of the larynx are relatively uncommon, and oncocytic cysts are considered particularly rare, as is borne out by the numerous reports of single cases and small series Of 22 publications, only 5 have dealt with more than 2 cases (Table I) However, in a 20-year material from the Mayo Clinic, De Santo et al (1970) observed that 35% of all laryngeal cysts were lined with oncocytes or contained these cells; they emphasized the uncharacteristic clinical appearances of this histological variant. That our 5 cases occurred within a period of 2 months must be ascribed to chance On revision of laryngeal cysts from the period 1969–74, a total of 13 cases, 2 could be re-classified as oncocytic cysts. Thus, out of a total of 18 laryngeal cysts, 7 (or 39%) were oncocytic When also considering the findings of De Santo et al., this suggests a higher incidence than is generally assumed. The two added cases did not differ from the other 5, clinically or morphologically. Both patients were women, aged 62 and 80, and the cysts affected the false cord and the ventricle respectively.

RÉSUMÉ

Les kystes onkocitiques constituent un sous-groupe anatomo-pathologique bien défini parmi les formations kystiques du Larynx Ils proviennent la plupart des fois des ventricules laryngée, et se développent chez les personnes d âge moyenne et les vieux Cliniquement ces tumeurs n'ont pas de caracteristiques speciales De tels kystes sont interpretés plutôt comme kystes de rétention que de véritables néoplasies La question de sa rarité se discute ayant comme base 5 cas diagnostiqués dans une période de 2 mois

ZUSAMMENFASSUNG

Die oncocytischen Cysten bestehen aus einer pathologisch anatomisch wohldefinierten Untergruppe der cystischen Bildungen des Larynx Sie gehen meistens vom Larynxventrikulus aus und werden bei Personen im mittleren und älteren Alter gefunden Klinisch sind diese Tumoren uncharakterisch Oncocytische Cysten sollen als Retentionscysten aufgefaßt werden und nicht als ein wahres Neoplasi Die Frage ihrer Seltenheit wird auf Hintergrund von 5 Fällen diskutiert, die in einer Periode von 2 Monaten diagnostiziert sind

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COMBINED THERAPY FOR UNDIFFERENTIATED GIANT AND SPINDLE CELL CARCINOMA OF THE THYROID

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Abstract Undifferentiated giant and spindle cell carcinoma is an unusual and high grade malignant type of cancer of the thyroid The results of surgical and/or radiotherapeutic treatment have been discouraging Only a few patients respond to therapy, usually for a short time Recently, a few reports have been published on the effect of chemotherapy or a combined treatment of surgery, radiotherapy and chemotherapy In the present paper the results of combining radiotherapy and chemotherapy, 5-FU and cyclophosphamide are reported For the future a more aggressive approach is discussed The therapy should include surgical resection of the main bulk of the tumour followed by radiotherapy and concomitant chemotherapy and thereafter prolonged adjuvant chemotherapy

Undifferentiated giant and spindle cell carcinoma of the thyroid is a rare disease, and it only accounts for 6–10% of all malignant tumours of the thyroid It is a disease of advanced age, the patients usually being about 70 years old Often the patients have a long history of preceeding benign thyroid disease, and microscopic examination of thyroidectomy specimens has shown concomitant well differentiated follicular or papillary carcinoma in 25–80% of patients with undifferentiated giant and spindle cell carcinoma of the thyroid (Nishiyama et al 1972) It has been assumed that this type of undifferentiated carcinoma represents a transformation of a longstanding well differentiated carcinoma (Hutter et al, 1965, Ibanez et al 1966 Beemer & Baker, 1970)

The tumours are usually unresectable, and their radiosensitivity is considered to be low So far the experience of chemotherapy is limited (Harada et al, 1971, Gottlieb et al, 1972, Gottlieb & Stratton Hill, 1974) No generally accepted method of therapy seems to exist The prognosis has been reported to be extremely poor, and there are few malignant diseases where a fatal outcome has been so inevitable (Silverberg et al, 1970) The immediate cause of death is usually local progression of the thyroid tumour

In recent years the results of combined therapy with surgery, radiation therapy and chemotherapy in undifferentiated giant and spindle cell carcinoma of the thyroid have been reported One of the first was given in autumn 1972 by Wallgren & Norin (1973) They reported a patient who relapsed locally soon after surgery The patient was then treated with radiation therapy, but the tumour continued to progress Concomitant chemotherapy with 5 fluoro-uracil (5 FU) and cyclophosphamide was given, and a complete local remission was obtained The patient later died from generalization of the disease

Impressed by this good local effect we decided in 1973 to use this type of treatment in patients with undifferentiated giant and spindle cell carcinoma of the thyroid The purpose of the present paper is to report the technique used by us and early results

Table I *Symptoms at presentation in 8 patients with undifferentiated giant and spindle cell carcinoma of the thyroid*

Symptoms at presentation	Number of patients
Tumour	8
Pain	2
Hoarseness	1
Dyspnoea	3
Dysphagia	1

MATERIAL AND METHODS

The series includes 8 patients who were treated during the years 1973–1975 for undifferentiated giant and spindle cell carcinoma of the thyroid Three of the patients were treated during the summer of 1975 which gives a relatively short possible follow up for the whole series

Three of the patients were males and 5 were females and their ages ranged between 54 and 90 years (mean 71 years)

Three of the patients had a history over many years for benign disease of the thyroid

The length of history for malignant tumour of the thyroid was usually short being less than 2 months in 4 patients, 2–4 months in 3 and 6 months in 1 patient The symptoms at presentation appear in Table I All patients had a large tumour in the thyroid, and this was usually the symptom that had urged the patient to seek medical advice The other symptoms mirror the pronounced tendency of the tumour to invade neighbouring structures

In all the patients scintigraphy of the thyroid showed a markedly reduced uptake and in all patients the scintigraphic image was compatible with carcinoma of the thyroid

Roentgenographic examinations showed in all patients a slight to moderate dislocation or compression of the trachea Pulmonary metastases were demonstrated in 1 of the 8 patients before commencing therapy

The general condition was markedly affected in 3 patients Three patients had a slight leucocytosis, but otherwise the peripheral blood picture showed no changes The ESR was elevated in all patients with a mean of 60 mm/1 hr

The diagnosis was established in all patients by means of fine needle aspiration biopsy Aspirations were performed from different parts of the tumour The material was stained

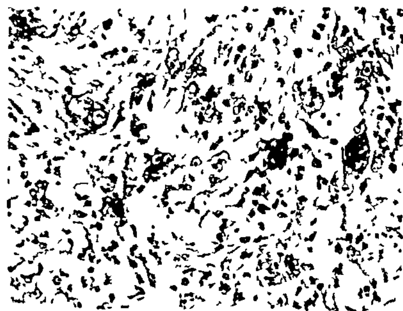


Fig 1 A The pleomorphic appearance of a giant and spindle cell carcinoma of the thyroid ×250

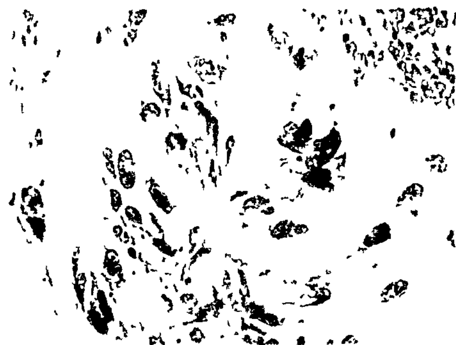


Fig 1B Fine needle asp ration biopsy smear from the same case as in Fig 1A ×400

with haematoxylin eosin and according to May Grunwald Giemsa In 2 patients biopsy material for histology was also available (in 1 patient a biopsy and in 1 patient thyroid ectomy material) The histologic and the cyto logic appearance of undifferentiated giant and indle cell carcinoma of the thyroid in one the patients appear in Fig 1

It was possible to perform a thyroidectomy in 1 patient All 8 patients were treated with combined radiotherapy and chemotherapy (Table II) The radiation therapy was given with Cobalt 60 and two opposed fields The target included the thyroid and the regional lymph nodes in the neck supraclavicular fossae and the upper part of the mediastinum A central absorbed dose of 4800 rad in 20 frac tions in 28 days was aimed at The chemo-therapy consisted of 5 FU and cyclophospha mide The 5 FU was given intravenously as an infusion over 12 hours starting 20 hours before and ending 8 hours before fractions number 1, 3, 5 7 and 9 with 0 5 g each time= totally 2 5 g The cyclophosphamide was given with 0 2 g daily for the first 10 fractions= totally 0 2 g

RESULTS

Five patients received a full course of radia tion therapy whereas the radiation treatment was interrupted at a lower absorbed dose in 3 patients because of deteriorating general condition or the presence of distant meta stases For similar reasons 2 patients only received reduced doses of chemotherapy whereas the full chemotherapy was given to 6 patients

All patients experienced slight but well tolerable mucositis and skin reactions Bone marrow toxicity presented no problem In no patient was the radiation therapy or chemo-therapy modified because of therapy related reasons

A complete remission was seen in 1 patient (Table III) and a partial in 1 The tumour stayed unchanged in 4 but progressed in 1 In 1 patient who had a thyroidectomy, the effect of radiotherapy and chemotherapy was con sidered not evaluable but the thyroid tumour did not reappear during the 5 month follow up

Two of 5 patients (K L and K N) who received a full course of combined radiation

Table II *Combined radiotherapy and chemotherapy in undifferentiated giant and spindle cell carcinoma of the thyroid*

Radiotherapy
Cobalt-60 2 opposed fields central absorbed dose=4 800 rad/20 fractions/28 days

Chemotherapy
5 FU [illegible]
[illegible] total[illegible]
Cyclophosphamide 0 2 g i v on days of fractions 1 through 10=totally 2 0 g

therapy and chemotherapy are alive after 26 and 5 months respectively (Fig 2), whereas 3 patients (R K, E B and H P) have died Patient K L had a complete regression of the tumour and patient K N has not relapsed after thyroidectomy In patients R K, E B and H P the tumour stayed unchanged, but the patients died from generalized disease Of the 3 patients who did not receive a full course of combined therapy, 1 (E G) had a partial regression of the tumour but died in deteriorating general condition without demonstrated metastases, 1 (H K) did not show any change in size of the tumour and died from generalized disease, and 1 (A J) died from locally progressive disease

Thus, of all 8 patients 2 are alive (Fig 2) after 26 and 5 months respectively without evidence of disease, whereas 6 have died within 4 months, 4 of them having shown distant metastases

DISCUSSION

Due to progression of local growth and pressure symptoms, causing dyspnoe and dysphagia, patients with undifferentiated giant and spindle cell carcinomas are often admitted to head and neck units

In the present material these symptoms were also found The material also agrees in frequency, age and sex incidence with other reports (Rafla, 1969, Beemer & Baker, 1970)

Fine needle aspiration biopsy has been used as a diagnostic method in this material This method has also been used by Löwhagen & Springer (1974) and Jereb et al (1975) and is considered reliable for diagnosis Using this technique multiple aspirations from different parts of the tumour can be done easily This is contrast to open excisional biopsy where only one or two parts of the thyroid will be examined In undifferentiated giant and spindle cell carcinoma some areas of well-differentiated thyroid carcinomas may occur (Wychulis et al, 1965, Ibanez et al, 1966, Silverberg et al, 1970) Thus, there is a risk that if only one or two biopsies are taken, only well-differentiated parts are biopsied, giving an incomplete diagnosis

The poor prognosis in undifferentiated giant and spindle cell carcinoma has been discussed by Woolner (1971), Franssila (1975) and Russell et al (1975) This course of the disease mirrors the insufficient therapy Recently, Halnan (1975) stressed the need for new lines or alterations in the treatment The immediate problem in the disease is local growth and recurrences The concomitance of well-differentiated cancer has led to recommendations that all well-differentiated thyroid carcinomas are to be operated with a total thyroidectomy Complete surgery will eliminate multicentric foci of well-differentiated thyroid carcinoma left at a subtotal operation which otherwise may transform into undifferentiated giant and spindle cell carcinomas (Frazell & Foote, 1958, Ibanez et al, 1969, Nishiyama et al, 1972, Kyriakides & Sosin, 1974)

Table III *Effect of combined radiotherapy and chemotherapy on the thyroid tumour in 8 patients*

	Number of patients
Complete regression	1
Partial regression	1
Unchanged	4
Progression	1
Not evaluable	1

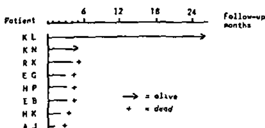


Fig 2 Follow up of 8 patients with undifferentiated giant and spindle cell carcinoma of the thyroid after combined therapy

In accordance with modern principles of tumour treatment the main bulk of the tumour should be removed before radiotherapy However, in undifferentiated giant and spindle cell carcinoma this is possible in only 20% of the patients (Nishiyama et al, 1972)

In summarizing the therapeutic results in anaplastic thyroid carcinoma Rafla (1969) stressed the fundamental role of radiotherapy He pointed out that any method strengthening radiotherapy is worth trying Combined therapy of 5-FU and radiotherapy used in our material and this mode of tumour synchronization has been reviewed by Vermund & Gollin (1968) 5-FU inhibits the thymine synthesis, [illegible] to a less stable DNA molecule which more sensitive to irradiation In squamous carcinoma of the oral cavity the combination of 5-FU and radiotherapy had an enhanced antitumour effect (Gollin et al, 1972) In transplanted leukemic cells in mice Vietti et al (1971) found that synergistic effect was obtained when 5-FU was given 14–24 hours preceeding radiotherapy as well as when 5-FU was given 8 hours after radiotherapy The former interval is in agreement with the interval used by us

No methods of choice for chemotherapy in undifferentiated giant and spindle cell thyroid carcinoma exist Recently, some papers have been published on the ultra structure of this tumour but they give no clue to therapy (Jao & Gould 1975 Goal et al, 1975) The cells were seen dissociated and the cell surface displayed an enhanced activity in numerous microvilli and large extensions engulfing other neoplastic cells There was a well-developed granular endoplasmic reticulum

In single drug therapy of undifferentiated giant and spindle cell carcinoma some cases have responded Gottlieb et al (1972) noted a partial response in one patient treated with a daily low dose of methotrexate until toxicity After a relapse 11 months later, adriamycin was given with a good partial response for at least 12 months In a later study, Gottlieb & Stratton Hill (1974) noted a partial response in 2 patients out of 9, treated with adriamycin Harada et al (1971) treated four patients with bleomycin and in one case noted a good effect In a combined program of methotrexate and radiotherapy Jereb et al (1975) gave intra arterial or systemic a low dose of methotrexate daily to five patients until toxic symptoms The therapy was combined with radiotherapy In all cases the undifferentiated tumour regressed, in four patients completely However, due to severe complications the therapy was discontinued and then all tumours re curred

Rogers et al (1974) have tried another modality combining surgery, radiotherapy and chemotherapy They found this combination more effective than other therapeutic methods After resection of tumour, Cobalt-60 therapy was started Treatment with actinomycin-D was started after 4 weeks of radiotherapy, and continued as repeated doses, even after radiotherapy Three of the 6 patients were living free of disease more than 2 years

For local control of tumour growth the recommendations by Wallgren & Norin (1973) have been followed The local results may be considered to be good and there were no complications necessitating discontinuation of therapy However, this local therapy may be combined with other modalities Gross resection of tumour will facilitate the effects of radiotherapy Halnan (1974) has stressed as a worthwhile trial prophylactic intermittent intensive multiple drug therapy This thera

peutic modality may be suitable after local elimination of tumour

All cases must be substituted with thyroid hormones though no hypothyreotic state has been demonstrated (Rafla, 1969, Kyriakides & Sosin 1974) MacGregor & Ham (1972) have reported a case of undifferentiated giant and spindle cell carcinoma treated with thyroid ectomy and 3 grains of desiccated thyroid extract and alive 6 years after treatment started

ZUSAMMENFASSUNG

Das undifferenzierte Riesen und Spindelzellkarzinom der *Schilddruse ist eine ungewohnliche aber hochmaligne* Form der Schilddrusenkarzinome Chirurgische Therapie und Radiotherapie haben bisher schlechten Erfolg gehabt *Wenn eine Wirkung der Therapie zu beobachten* war war sie gewöhnlicherweise von kurzer Dauer In den letzten Jahren sind einige Arbeiten uber die Wirkung *der Chemotherapie oder einer Kombination von Chirur*gie Radiotherapie und Chemotherapie veroffentlicht worden In der vorliegenden Arbeit werden die Be*handlungsresultate* einer von Radiotherapie und Kombination Chemotherapie 5 Fluorourazil und Cyklophosphamid bei diesen Tumoren berichtet Eine zukunftige aggressivere Therapie wird diskutiert Diese Therapie sollte aus folgenden Momenten bestehen chirurgische Entfernung einer moglichst großen Tumormasse anschließend simultan Radiotherapie und Chemotherapie und danach zusatzlich wahrend langerer Zeit Chemotherapie

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MESSUNG DES SCHLEIMHAUTTRANSPORTES IN MENSCHLICHEN NASE MIT ^{51}Cr MARKIERTEN HARZKUGELCHEN

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Abstrakt Der Mucocılıartransport der menschlıchen Nase wurde mıt sehr kleınen Harzpartıkeln untersucht welche durch radıoaktıves ^{51}Cr beladen waren Dıe von den vor*hergehenden Untersuchern* verwendete Methode wurde ın wesentlıchen Punkten modıfızıert Dıese *Modifıkatıonen* betraf Art des Isotopes Große der Teılchen *pH Applı*katıonstechnık Meßtechnık Vermınderung der lokalen *Strahlenwırkung* und schlıeßlıch Schaffung der Moglıchkeıt exakte Transportgeschwındıgkeıts*messungen* nıcht nur ın horızontaler sondern auch ın vertıkal*er und* schräger Rıchtung Keın Mucocılıartransport wurde ın *F nf von* [illegible] hauttransport Es wurde weıters an gesunden Propanden *der Eınfluß* untersucht welcher durch homolateralen oder kontralateralen experımentellen *Verschluß* eıner Nasenhalfte sowıe durch Tabakrauch verursacht *wırd* Patıenten mıt verschıedenen Krankheıten Pollenallergıe ım anfallsfreıen *Intervall* chronısche Rhınıtıs Septumdevıatıon und perforatıon und Status *post* Laryngektomıe wurden ebenfalls studıert

Dıe Fahıgkeıt der Nase neben anderen Funktıonen dıe eıngeatmete Luft von den grobsten Verunreınıgungen zu befreıen tragt wesentlıch zur Erhaltung der Gesundheıt des Menschen beı

Der Stand unseres Wıssens der Schleımhauttransportfunktıon entstammt Tıerversuchen welche teıls ın vıtro (Proetz, 1953 Mercke et al , 1974) teıls ın vıvo (Dalhamn & Rylander, 1962, Kreuger et al , 1959) durchgeführt worden waren

Dıe ersten Beobachtungen des Mucocılıartransportes am lebenden Menschen stammen von Yates (1924) und Hıldıng (1931) Messerklınger beobachtete (1951) ın vıvo mıt Fluoreszenzfarben den Sekretstrom auf der menschlıchen Schleımhaut der oberen Luftwege und eroffnete uns mıt seınen ın vıtro Versuchen an der menschlıchen Leıche (1966 1967) eınen vollıg neuen Eınblıck ın den Schleımhauttransport der bıs zu 24 Stunden den Herzstıllstand uberlebenden Schleımhaut der Nasennebenhohlen Van Ree & van Dıshoeck (1962) untersuchten als erste Transportgeschwındıgkeıten ohne Lokalanasthetıca und Ewert (1965) lıeferte uns erste genaue Messungen des Schleımhauttransportes des vorderen Anteıls des Septums

Eıne kontınuıerlıche Verfolgung eıngebrachter Partıkel entlang der Nasenschleımhaut bıs zum Epıpharynx war erst durch dıe Verwendung radıoaktıver Tragersubstanzen ın Verbındung mıt geeıgneten Messgeraten möglıch (Proctor et al 1965, 1973, Quınlan et al 1969, Andersen et al , 1971, 1972, 1974 1975 Sakakura et al 1973) Dıe von dıeser Arbeıtsgruppe gewonnenen Erkentnısse zeıgten eındrucksvoll dass Versuche dıe am lebenden

Dıese Arbeıt wurde ausgefuhrt mıt Unterstutzung des schwedıschen medızınıschen Forschungsrates (Projekt 749)

Menschen durchfuhrbar und zumutbar sind den hochsten Informationsgrad besitzen

So stiessen ihre Beobachtung des unverminderten Schleimhauttransportes unter langdauernder Trockenheit in einer Klimakammer (Andersen et al , 1974) alle bisherigen Erkenntnisse auf diesem Gebiet um

Die bis jetzt als Trägersubstanz verwendeteten Harzpartikel mit einem Durchmesser von 0,5 mm erschienen uns hinsichtlich Grösse und Gewicht eine unphysiologisch grosse Belastung der Flimmerzellen zu sein Nimmt man wesentlich kleinere Harzkügelchen mit einem Durchmesser von 0,01–0,05 mm und belegt damit eine kleine Flache der Nasenschleimhaut so ist zu erwarten, dass die ciliaren Transportkrafte weniger stark negativ beeinflusst werden Weiters sollte Sorge getragen werden, dass die eingebrachten Partikel mit einem optimalen pH Wert (6,7–7,2) auf der Schleimhaut zu liegen kommen, da Einflusse auf Sekretbeschaffenheit und damit auch auf Transportgeschwindigkeiten zu bestehen scheinen (Breuninger, 1964) Ein wesentlicher Faktor in unserem Bemühen, mit Hilfe dieser Methode eine weitestgehend exakte Information von der Schleimhauttransportfunktion der menschlichen Nase in vivo zu bekommen, war die Vermeidung jeglicher Strahlenschadigung und die Beschrankung der Strahlenbelastung auf ein Minimum

Aus diesem Grunde war das von vielen Autoren verwendete $^{99}Tc^{m}$ nicht gestattet als Trägersubstanz der Harzpartikel zu verwenden, da von ihnen ein hoher Anteil Elektronenbestrahlung auf die darunterbefindlichen Zellkerne der Nasenschleimhaut trifft Dieser Anteil von Strahlenbelastung wird naturgemass hoher je langsamer sich das radioaktive Partikel weiterbewegt Wir stellten uns daher zur Aufgabe, diese Methoden wesentlich mehr den physiologischen Verhaltnissen anzupassen neuen radiophysikalischen Erkenntnissen Rechnung zu tragen und den weiteren Fortschritt in der Apparatetechnik uns zu Nutze zu machen Als Trägersubstanz verwendeten wir aus diesen Grunden ^{51}Cr in Form des Na CrO_4^-, das mit Hilfe eines jonenaustauschenden Harzes an dieses gebunden wird

Die Messtechnik welche Proctor & Wagner (1965), Quinlan et al (1969), Sakakura et al (1973) und Andersen et al (1975) anwendeten, ermoglichte nur genaue Messungen der Transportgeschwindigkeit, wenn das radioaktive Harzkügelchen sich im rechten Winkel zu den Kollimatorschlitzen bewegte Jeder schrage oder gar vertikale Transportverlauf musste langsamere Werte ergeben

Unter diesen Vorbedingungen und aufbauend auf schon gewonnene Erkenntnisse, stellten wir uns folgende Fragen

1) Wie gross ist die normale Transportgeschwindigkeit der Nasenschleimhaut?
2) Ändert sich der Schleimhauttransport, wenn man wahrend der Untersuchung die zu messende bzw die kontralaterale Nasenhalfte verschliesst?
3) Beeinflusst Tabakrauch, welcher wahrend der Messung durch die Nase ausgeatmet wird die Transportgeschwindigkeit?
4) Haben Nasentropfen mit schleimhautabschwellender Wirkung auch Einfluss auf den Mucociliartransport?
5) Wie ist die Schleimhauttransportfunktion bei Pollenallergikern in allergenfreien Jahreszeiten (Herbst)?
6) Ist es technisch möglich, die Geschwindigkeit der eingebrachten radioaktiven Harzpartikel nicht nur etappenweise sondern vielmehr kontinuierlich zu registrieren und diese auch in Relation zur tatsächlichen Transportrichtung zu setzen?

MATERIAL UND METHODE

An 35 Personen (22 Frauen und 13 Manner) im Durchschnittsalter von 27,8 Jahren (zwischen 19 und 46 Jahren sowie ein Pat mit 65 Jahren) wurden meist zweimal im Abstand von einigen Tagen immer zur gleichen Tageszeit die Messungen durchgeführt Davon waren 5 Pollenallergiker (Durchschnittsalter 25,8 Jahre) und 1 Patient mit Stat p Laryngek-

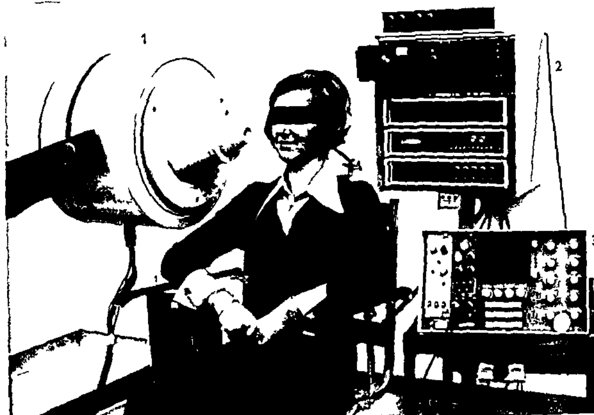


Abb 1 Untersuchungsperson wahrend der Messung Gammakamera (*1*) Photoskop m t Polaro dkamera (*2*) Multichannel Analyzer (*3*)

tomie (65 a) an welchem nur jeweils eine Mes sung durchgeführt wurden Insgesamt wurden 53 Messungen erzielt Die Messungen wur den im Zeitraum August bis September 1975 im Radiophysikalischen Labor der Universi tats Klinik Uppsala durchgeführt Die Raum temperatur schwankte zwischen 22° und 25°C Luftfeuchtigkeit von 50–64% rel Feuchtig keit

Farbung und Beladung der Partikel

Dowex Partikel (1 SBR×8 Partikelgrosse 0 01–0 05 mm) wurden in jonenfreiem Wasser mit Bromphenolblau (pH Umschlagspunkt 3 0–4 6) gefarbt Von diesem Wasser Partikelge misch wurde mit einer Spritze ca 40 μl ent nommen und in eine Glaspipette gespritzt welche mit einem einfachen Teefilter an der Spitze versehen war Unter leichten Sog e stand an der Spitze eine 3 mm lange und 1 n dicke Saule von Jonenaustauschharz Un fahr 15 μl wassrige ^{51}Cr Chromatlosu (CJS 1 5 mCi/ml Radiochemical Cent Amersham G B) wurden hinzugefugt und c nach mehrmals mit Jonenfreiem Wasser waschen Die beladenen Partikel wurden dann auf eine mit Filterpapier umwickel ellyptisch geformte und mit Natriumph phat Puffer getrankte Stahlnaden gebrac Durch Verwendung dieses Natriumphosph Puffers erhalten die Partikel den gewunscht pH von 7 0 Die Partikel hafteten in Fo eines winzigen Kegels (Basisdurchmesser mm) auf dem feuchten Filterpapier und w den so bis zur Anwendung in einem v schlossenem Glaschen aufbewahrt

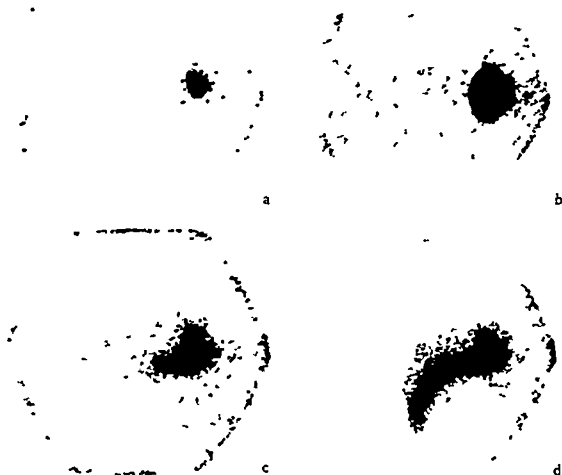


Abb 2 Transportspur vom Bildschirm aufgenommen Nasenspitze rechts (*a*) Partikel nach Applikation (*b*) Die Partikel haben den Unterrand der unteren Muschel erreicht (*c*) Weiterbeförderung entlang der unteren Muschel nach dorsal (*d*) Die Partikel haben den Nasopharynx passiert (Knick nach unten) und bewegen sich entlang der Rachenseitenwand nach caudal

Applikation und Messtechnik

Die Testperson sass auf einem stabilen mit Kopfstütze versehenem HNO Untersuchungsstuhl Bei den uns zur Verfügung gestandenen kooperativen Untersuchungspersonen war eine weitere Fixierung unnötig Eine Gamma Kamera (Radicamera Selektronik Danmark) mit einem Pinhole Kollimator (Ø 5 mm) wurde in die Mitte der horizontal gehaltenen Linie zwischen Gehörgang und Nasolabialwinkel gebracht (Abb 1) Der Abstand des Kollimators von der sagittalen Medianlinie betrug konstant 11 cm Die auf dem Applikator befindlichen ^{5} Cr beladenen Harzpartikel wurden sodann unter direkter Sicht auf die mediale Seite der untere Muschel 1 cm von deren Ansatz gebracht In einigen gesondert beschriebenen Fallen wurden die Partikel auf die dieser Applikationsstelle gegenuberliegen den Septumschleimhaut gebracht

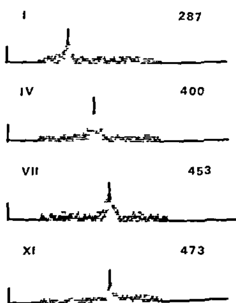


Abb 3 Ausschnitt aus dem Messvorgang am X Kanal des Multichannel Analyzers Die ⁵¹Cr markierte Partikel ansammlung passiert nach der 1 Minute Schlitz Nr 287 nach der 4 Minute Schlitz Nr 400 nach der 7 Minute Schlitz Nr 453 und nach der 11 Minute Schlitz Nr 473
Es wird jeweils nach 25 sec ein Messwert im X Kanal und nach weiteren 25 sec ein Messwert im Y Kanal notiert

Die tatsachlich in die Nase gebrachte Radioaktivitat wurde in einem Radioisotope Ca (Capintec) durch Messung des Applikators vor und nach Einbringung der Probe ermittelt Wurde kein Partikeltransport beobachtet bzw verblieben die Partikel zu lange im Nasopharynx so wurden sie instrumentell entfernt

Messung des Schleimhauttransportes

Der Transport der Harzpartikel von der Applikationsstelle bis in den Nasopharynx wurde optisch und photographisch sowohl am Photoskop als auch am Leuchtschirm der Gamma Kamera verfolgt (Abb 2) Die Bewegung der Partikel entlang der Sagittallebene wurde abwechselnd in X und Y Signalen zerlegt und mit Hilfe eines Multichannel analyzer (Nuclear Data) aufgezeichnet Zur Bestimmung jeweils einer X bzw Y Position wurde pro Minute eine Messperiode von 25 sec benotigt In der Regel zeigte die Aktivitat eine scharfe Spitze im Oszilloskop sodass direkt der entsprechende Kanal bestimmt werden und dessen Nummer entsprechend dem X bzw Y Kanal digital abgelesen werden konnte (Abb 3)

Das Messystem wurde kalibriert indem ^{51}Cr Punkt Aktivitaten auf einer zur zentralen Achse der Gammakamera orthogonalen Oberflache in Beziehung zum Abstand des Kollimators von der sagittalen Medianlinie der Testperson gesetzt wurden

Die jeweilige X und Y Position sowie die Kalibrierung wurden mit Hilfe eines Tischrechners (Hewlett Packard) in die effektiven Transportdaten umgewandelt

Die statistischen Berechnungen wurden mit dem Mann Whitney U Test (Siegel 1956) durchgefuhrt

Ergebnisse der Applikationstechnik

Der Beladungsvorgang war sowohl effektiv als auch ausserordentlich reproduzierbar

Ungefahr 2‰ der Harzpartikelsaule zugefuhrten Aktivitat fand sich in der Filtrierflussigkeit Die durchschnittliche Aktivitat am Applikator betrug 55 μCi mit einer Standardabweichung von ± 5μCi In die Nase wurden durchschnittlich 30 μCi eingebracht mit einer Standardabweichung von ± 6 μCi Die Partikel bedeckten auf der Nasenschleimhaut eine Flache von ca 10 mm² Bei den meisten Versuchen blieb dieser Teppich von Partikel uber die ganze Wegstrecke bis zum Nasopharynx zusammen In einigen Fallen teilte sich diese Partikelansammlung in zwei Teile wobei es auch hier gelang die manchmal mit verschiedener Geschwindigkeit transportierten Partikel zu registrieren (Abb 4)

Der Messgenauigkeit am Multichannel Analyzer betrug ± 1 mm

Ergebnisse der Schleimhauttransportmessung

An 21 Probanden wurden Messungen mit unbeeinflusster Nasenatmung durchgeführt Die Durchschnittstransportgeschwindigkeit

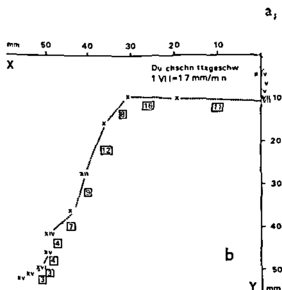


Abb 4 (*a*) Photoskopaufnahme des Partikeltransportes über den gesamten Zeitraum von 19 Minuten Man sieht dass sich die Partikel von Beginn des Transportes in zwei Portionen geteilt haben und verschiedenen Trans portwegen folgen (*b*) Transportdataanalyse im X Y Diagramm von Verlauf und Geschwindigkeit des in Abb 4*a* abgebildeten langeren (oberen) Transportweges Nach anfänglich (1 –8 Minute) langsamen vertikalen Transport von der Applikationsstelle nach caudal mit einer Durchschnittsgeschwindigkeit von 1 7 mm/min erhoht sich die Geschwindigkeit von der 8 bis zur 9 Minute auf 16 mm/min Die in den Messzeitraumen 9 –18 Minute errechneten effektiven Geschwindigkeiten sind eingerahmt

von Beginn einer zu beobachtenden Bewegung bis zum Ende des Transportes im Nasopharynx betrug 3,6 mm/min

Um ein intraindividuelles Korrelat der Ver suche zu bekommen, wurden bei den Ver suchen mit experimenteller Anderung der Verhaltnisse in Nasenlumen zuvor Messungen an derselben Versuchsperson ohne jeden Einfluss durchgeführt Wahrend des ganzen Versuches hatte der Proband durch die Nase zu atmen

Um zu prufen welchen Einfluss eine erhöhte Nasenatmung und ein völliger Stop der Nasenatmung hat, verschlossen wir an 5 gesunden Versuchspersonen die kontralaterale Nasenhalfte und erhöhten dadurch den Atemstrom in der zu messenden Seite Bei 3 anderen Probanden verschlossen wir nach Einfuhrung der Aktivitat den Naseneingang der zu messenden Seite mit einem Pflaster In allen 8 Fallen wurden zur selben Tageszeit einige Tage zuvor Messungen bei unbeeinflusster Nasenatmung durchgefuhrt

Die Durchschnittsgeschwindigkeit in der Gruppe mit erhöhten Atemstrom betrug 3,9 mm/min Dagegen fand sich in der Gruppe mit Atemstop in der zu messenden Seite eine Durchschnittsgeschwindigkeit von 6,6 mm/min Der Unterschied ist statistisch nicht signifikant

Die Beobachtung der Durchschnittsgeschwindigkeit gab jedoch nur einen ausserst groben Uberblick über das Transportverhalten der Schleimhaut Dies war letztlich nichts anderes als ein Screen test wie ihn Proctor mit Sacharin beschrieb und wie man schon vor Jahrzehnten mit Farbpartikel die Transportgeschwindigkeit in der Nase bestimmt hat, indem man die Zeit zwischen Start am vorderen Nasenanteil und Wiedererscheinen im Nasopharynx Spiegel gestoppt hat

Der echte Vorteil dieser verfeinerten Messtechnik liegt in der Moglichkeit das Transportverhalten in Abhangigkeit von Richtung und Geschwindigkeit zu registrieren

Wahrend in den Arbeiten von Andersen, Quinlan und Proctor maximal 6 vertikale Kol limatorschlitze den Partikeltransport durch Mittelung messen konnten, wurde bei unseren Messungen kontinuerlich in Minutenabstand auf Millimetergenauigheit der aktuelle Stand der Partikel erhoben Hinzu kommt noch ein weiterer wesentlicher Fortschritt Die oben beschriebene Messmethode ist nur dann in der Lage exakte Geschwindigkeiten zu ermitteln, wenn die Partikel sich exakt im rechten Win-



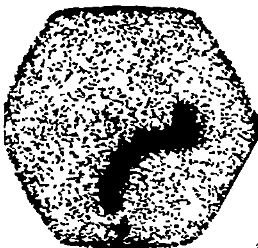
Abb 5 Bilder von verschiedenen Transportverläufen (*a*) Langsamer Transportbeginn von der Applikationsstelle Schnellerer Transportverlauf im mittleren Anteil (zu erkennen an der zarteren Grautönung) und Stop im Nasopharynx (*b*) Sehr schneller und kontinuierlicher Transportverlauf bis uber den Nasopharynx hinaus Der Pfeil weist auf Partikel die bei der Applikation in den Vibrissae hängen blieben und nach Ende der Untersuchung entfernt wurden (*c*) Langsamer und kontinuierlicher Transportverlauf entlang der unteren Muschel bis an der Vorderrand eines in die Muschel ragenden Septumdornes

kel zu den Schlitzen weiterbewegen Verlauft der Schleimhauttransport aber nach caudal oder kranial bzw in irgendeiner zu den Messchlitzen schrägen Achse so mussten mehrere Geschwindigkeiten als tatsachlich vorhanden erhoben werden

In Ubereinstimmung mit den erwahnten Autoren fand sich in der Regel ein sehr langsamer Transportbeginn In einigen Fallen verblieben die Partikel bis zu 5 min an Ort und Stelle liegen um dann zum Teil mit hoher Geschwindigkeit weitertransportiert zu werden (Abb 5*a*) Die Partikel bewegten sich an der Applikationsstelle teilweise einige mm nach oben und vorne Diese Bewegung war jedoch ausserst langsam Trat einmal ein richtiger Schleimhauttransport ein so verlief er meist von der Applikationsstelle 1 cm dorsal des Muschelansatzes zuerst caudal bis an den Rand der unteren Muschel und diesem Rand entlang nach dorsal (Abb 5*b*) oder die Partikel kamen am Nasenboden zu liegen und bewegten sich hier entlang nach dorsal Bei Dev septi fand sich in drei Fallen das anfangs auf die untere Muschel gebrachte Partikelkonglomerat auf einen den hinteren Anteil der unteren Muschel genaherten Septumdorn wo dann auch der Schleimhauttransport stoppte (Abb 5*c*)

In den Abb 2*d* lasst sich deutlich ein Knick im hinteren Anteil des Schleimhauttransportes feststellen Hier wurden die Partikel in Richtung zum anterioren Wulst der Tuba Eustachii befördert In einigen Fallen beobachteten wir in diesem Bereich auch eine Weiterbewegung der Partikel zum posterioren Wulst der Tuba Eustachii

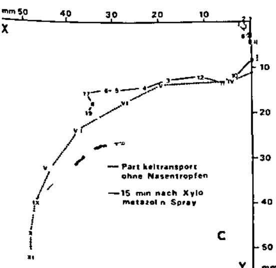
Eine anfanglich langsame Transportgeschwindigheit war keineswegs ein Indikator dass die Partikel auf ihrem weiteren Weg



a



b



nicht ausserst schnell (zwischen 10–16 mm/min) weiterbewegt wurden

Die Geschwindigkeit erhöhte sich meist im mittleren Anteil bis auf 16 mm/min (s Abb 4*b*) um dann weiter dorsal langsamer zu werden Der Transport ging aber nie kontinuierlich vor sich Schnellerer Transport wechselte mit langsamerem

Zusammenhang von Infekt der oberen Luftwege und Schleimhauttransport

Von den 21 Probanden mit experimentell unbeeinflusster Transportfunktion gaben 9 eine durchgemachten Infekt der oberen Luftwege (im Verlauf der letzten 10 Tagen) an oder hatten in der Untersuchung folgenden Woche eine Erkaltung Von 7 Fallen ohne jeden Schleimhauttransport gehörten 5 dieser Probandengruppe an Bei den übrigen 2 Probanden konnten keine erkennbare Ursache fur das völlige Fehlen eines Schleimhauttransportes erhoben werden

Die Durchschnittsgeschwindigkeit bei jenen 9 Probanden mit Infektanamnese betrug 1,7 mm/min Dieser niedere Wert erklärt sich schon daraus, dass wir an fünf von diesen neun Probanden überhaupt keinen Weitertransport der eingebrachten Partikel feststellen konnten

Transportgeschwindigkeit am Septum

An 5 Probanden wurde Transport richtung und -geschwindigkeit untersucht An zwei Probanden davon wurde eine durchschnittliche Geschwindigkeit von 2,2 mm/min registriert Bei drei Probanden wurde von Anfang an kein Transport beobachtet Anamnestisch erhoben wir an diesen drei Personen

Abb 6 (*a*) Partikeltransport ohne medikamentosen Einfluss (*b*) Dieselbe Versuchsperson nach lokaler Verabreichung von Xylometazolin-Nasenspray (*c*) Transport [illegible]

(Messpunkte in arabischen Ziffern 1–19 Minute) ist der Schleimhauttransport deutlich verlangsamt. Die Partikel bleiben schon im Nasopharynx liegen

einen durchgemachten Infekt der oberen Luftwege im Verlauf der letzten 10 Tage jedoch waren sie zum Untersuchungszeitpunkt beschwerdefrei Bei einem Probanden mit einer chronischen Rhinitis St p submucoser Septumresektion vor drei Jahren und einer bestehenden Septumperforation (Durchmesser ca 8 mm) stoppte der anfangs schon ausserst langsame Transport (0 6 mm/min) am Vorderrand dieser Septumperforation

Schleimhautabschwellendes Medikament – Xylometazolin chlorid und Transport an der unteren Muschel

Sechs Probanden ohne Erkaltungsanamnese 10 Tage vor der Untersuchung oder 1 Woche nach der Untersuchung wurden in die zu untersuchende Seite 12–15 min vor dem Versuch mit 0 1% Xylometazolin chlorid (Otrivin® Ciba) gesprayt Etwaige noch auf der Nasenschleimhaut uberschussige Flüssigkeit wurde unmittelbar vor Versuchsbeginn durch Ausscheuzen entfernt Die Schleimhaut war regelmassig stark abgeschwollen und in 4 von 6 Fällen sehr blass Vier der sechs Probanden stellten eine Kontrollgruppe in sich dar An ihnen wurde einige Tage vorher eine Kontroll[illegible]ung ohne Beeinflussung der Nasenfunk[illegible] durchgefuhrt Die Transportgeschwindigkeit war in allen diesen Kontrollmessungen hoher als nach lokaler Gabe des Xylometazolin Sprays (Abb 6) Die unter Otrivineinfluss erhobene Durchschnittsgeschwindigkeit betrug 1 3 mm/min An zwei dieser vier Probanden beobachteten wir unter lokalem Xylometazolineinfluss keinerlei Partikeltransport Die Durchschnittsgeschwindigkeit der zwei Probanden die keine Kontrollmessungen hatten betrug 1 7 mm/min

Die statistische Berechnung ergab $P<0\ 05$ sowohl für die Doppeluntersuchungen an 4 Probanden als auch für alle sechs Versuchspersonen in Verhaltnis zu der Kontrollgruppe

Einfluss von Zigarettenrauchen

Von den 35 untersuchten Personen waren 13 Raucher und 21 Nichtraucher Der Patient mit St p Laryngektomie beendete 3 Wochen vor der Operation den Zigarettenkonsum Die statistische Berechnung des Zusammenhanges von Schleimhauttransportgeschwindigkeit und und Rauchen ergab keine Signifikanz Aus diesem Grund wurde eine Pilotstudie mit direkter Belastung durch Tabakrauch wahrend des Messvorganges bei Gewohnheitsrauchern angeschlossen Vier Probanden rauchten wahrend der Messung eine filterlose starke Zigarette (Marke Philip Morris) Es wurde in dem Probanden gewohnten Zeitabstand der Rauch durch den Mund inhaliert und gefordert dass der Rauch langsam durch die Nase auszublasen sei Abgesehen von den Intervallen der Rauchinhalation war der Mund geschlossen Dabei fand sich bei zwei Probanden keinerlei Schleimhauttransport bei den restlichen zwei jedoch eine erhohte Transportgeschwindigkeit Statistisch gab es keine signifikante Differenz zu den ubrigen normalen Probanden

Transportgeschwindigkeit bei Pollenallergikern im beschwerdefrei Intervall (Herbst)

Es wurden dabei Patienten untersucht die unter Kontrolle der Allergieambulanz standen Es wurden 5 Patienten untersucht die alle seit mindestens 6–8 Wochen beschwerdefrei waren Die Patienten litten zur Zeit des Pollenfluges (Birken Graser u a) unter starker bis massig starker Rhinitis allergica Alle waren einem Desensibilierungsversuch in Laufe der letzten Jahre unterzogen worden und hatten teils massigen teils keinen Therapieerfolg konstatiert

An 2 Patienten fanden wir keinen registrierbaren Schleimhauttransport Die Durchschnittsgeschwindigkeit bei den verbleibenden 3 Patienten betrug 3 2 mm/min (statistisch nicht signifikant) Es fiel auf dass bei dieser Patientengruppe es wesentlich schwerer gelang die notwendige Menge von Partikeln auf die Schleimhaut zu bringen Die Schleimhaut war in der Regel viel feuchter

Schleimhauttransport in den ubrigen klinisch pathologischen Fallen

Zwei Patienten litten unter massig starker chronischer Rhinitis mit geringer schleimig ei triger Sekretion Wir fuhrten die Aktivitat in diesen Fallen auf jener Seite mit vermehrter eitriger Sekretion ein In beiden Fallen war der Schleimhauttransport vermindert

Ein Patient wurde drei Wochen zuvor einer totalen Laryngektomie mit einseitiger Neck dissektion wegen Larynxkarzinom unterzogen Vor der Operation erhielt der 65 jahrige Patient eine Vorbestrahlung von 3500 R Die Durchschnittstransportgeschwindigkeit uber den gesamten Messbereich betrug 8 4 mm/min

DISKUSSION

Die Grundkenntnisse uber den Schleimhaut transport wurden und werden wahrscheinlich auch in Zukunft durch in vitro oder in vivo Untersuchungen an Tieren und Leichen gewonnen Versuche am lebenden Menschen haben lediglich den Zweck als Erganzung zu solch prinzipiellen Studien zu dienen welche durch Untersuchungen am Tier oder an der menschlichen Leiche nicht geklart werden konnen Es ist verstandlich dass Ergebnisse welche am Tier gewonnen werden nicht zur Ganze auf den lebenden Menschen übertragen werden konnen Selbst gute Korrelationen bei in vitro Versuchen wie sie Guillerm et al (1971) und Mercke et al (1974) zwischen Schleimhauttransport und Luftfeuchtigkeit verzeichneten haben zum den lebenden Menschen keine Beziehung (Andersen et al 1974)

Gewisse Faktoren wie z B der Einfluss einseitiger Verschluss einer Nasenhalfte der Einfluss von Infekt der oberen Luftwege der Einfluss des Tabakrauches und von Nasentropfen muss daher am lebenden Menschen studiert werden wenn wir eine Antwort auf die tatsachlich bestehenden Verhaltnisse im Menschen haben wollen

Folgende Methoden zum Studium des Schleimhauttransportes am lebenden Menschen stehen zur Verfugung

1) Einbringung von Farbpartikeln in die Nase und Messung der Zeit bis zum Erscheinen im Nasopharynx (Hilding 1931)
2) Eine ebenso grobe Messmethode jedoch als Screen Test gut verwertbar ist die von Andersen et al (1975) beschriebene Einbringung von Sacharinpartikel in die Nase und Messung der Zeit bis zur subjektiven Registrierung eines susses Geschmackes
3) Beobachtung des Schleimhauttransportes am Septum mit dem Binocularen Mikroskop (Ewert 1965)
4) Verwendung radioaktiver Partikel (Proctor et al 1965 1973, Quinlan et al 1969 Andersen et al 1971 1972 1974 1975 Sakakura et al 1973 welche auf die Schleimhaut des Nasenlaumens gebracht werden und entlang deren gesamter Ausdehnung verfolgt werden konnen

Die Ergebnisse von Ewert konnten mit den grossen Untersuchungsserien von Proctor Quinlan Andersen und Sakakura nicht in Einklang gebracht werden insbesondere bezuglich des Einflusses von verschieden grosser Luftfeuchtigkeit Seit diese gross angelegten Untersuchungen in der Klimakammer zeigten dass die Luftfeuchtigkeit keinen wesentlichen Einfluss auf den Schleimhauttransport der oberen Luftwege hat erscheinen uns weitere Untersuchungen auf diesem Gebiet nicht notwendig Unsere Experimente wurden im Zeitraum August–September durchgeführt wahrend dessen wir im Untersuchungsraum für kontantes Klima sorgen konnten und auch geringe Unterschiede zwischen dem Klima im Freien und dem Untersuchungsraum bestanden

Die Methode welche Proctor et al mit $^{99}Tc^{m}$ verwendeten birgt das Risiko in sich eine sehr hohe Elektronenstrahlendosis auf die Kerne des Schleimhautepithels zu bekommen Dieses Risiko ist besonders gross wenn kein Schleimhauttransport beobachtet wird und die Partikel an Ort und Stelle liegen bleiben Dieses Risiko ist wesentlich geringer wenn man ^{5}Cr markierte Partikel verwendet Hiermit

kann die direkte Schleimhautbelastung wesentlich vermindert werden (S Appendix)

Wir trugen weiters Sorge, dass bei nicht vorhandenem Schleimhauttransport nach 10 Minuten die einbegrachten Partikel unter direkter Sicht entfernt wurden Aus den oben beschriebenen Gründen wurden an ein und derselben Versuchsperson nie mehr als zwei Untersuchungen durchgefurt

Weiter anderten wir die Methode dahingehend, dass wir viele kleine 0,01–0,05 mm grosse Partikel verwendeten, wahrend Proctor wesentlich grossere Partikel mit einem Durchmesser von 0,5 mm in der Nase applizierte Diese grossen Partikel halten wir fur eine un physiologische Belastung der Mucociliarfunktion Wir sorgten auch dafur, einen pH um 7,0 an den eingebrachten Harzpartikeln zu haben, um weitere lokale negative Einflusse auszu schliessen

Dem technischen Fortschritt trugen wir dadurch Rechnung, dass wir ein Messystem an wandten in welchem nicht nur horizontale Fortbewegung sondern auch jeder andersgerichtete Weitertransport in der Sagittalebene exakt gemessen werden konnte Das Auflösungsvermogen der Gamma-kamera war so t, dass selbst Schleimhauttransporte auf zwei verschiedenen Wegen gleichzeitig registriert und gemessen werden konnten Es gelang auch den Mucociliartransport entlang des anterioren oder posterioren Wulstes der Tuba Eustachii bis in den Oropharynx zu verfolgen In Verbindung mit der anterioren Rhinoskopie konnten wir die Ursache eines Transportstill standes am Septum in einer Septumperfora tion und die Ursache eines Transportstillstan des an der unteren Muschel *in einer in diese* ragenden Septumdornes lokalisieren

Unsere Ergebnisse bezuglich der Transport geschwindigkeit ergaben niederere Werte als jene von Proctor, Quinlan Andersen und Sa kakura Dies ist erstaunlich waren wir doch bemüht mit allen uns derzeit zur Verfügung stehenden Hilfsmitteln den Einfluss auf den Schleimhauttransport auf ein Minimum zu bringen, aber wahrscheinlich war die totale von uns benutzte Partikelmenge grosser Auch die Untersuchungsergebnisse von Ewert welche mit einer ganz anderen Messmethode erzielt worden waren, weisen im Durchschnitt hohere Transportgeschwindigkeiten auf Je doch muss erwahnt werden, dass die Messer gebnisse aller auf diesem Gebiet arbeitenden Autoren grosse Streuungsbreite aufweisen und verglichen mit unseren Messwerten statistisch nicht signifikant sind

Den Versuchen am Menschen gingen solche an narkotisierten Kaninchen voran, um neben Kalkulationen des minimalen Strahlenbedarfes auch deren Transportverhalten bzw geschwindigkeiten zu studieren Wir fanden da bei eine Durchschnittsgeschwindigkeit von 5,4 mm/min Weitere Untersuchungen er schienen uns wegen der vollig anderen anatomischen Verhältnisse und wegen der notwen digen Ruhigstellung der Tiere durch Narkose nicht sinnvoll

Der experimentell ausgeloste einseitige Stop der Nasenatmung auf der zu messenden Seite erhoht zwar im Durchschnitt die Geschwin digkeit, jedoch nicht signifikant Es muss aber vermerkt werden, dass auch bei einem Laryngektomierten bereits drei Wochen nach der Operation ein sehr schneller Mucociliar transport gefunden wurde

Neun Versuchspersonen in unserer Serie hatten einen Infekt der oberen Luftwege 10 Tage zuvor oder 1 Woche danach wovon 5 keinerlei Partikelweiterbewegung aufwiesen Eine ähnliche Beobachtung machte Quinlan et al (1969) In unserer Untersuchungsserie hat ten nur zwei Untersuchungspersonen einen In fekt nach dem Experiment und auch an ihnen war kein Schleimhauttransport beobachtet worden, obwohl sie keine klinischen oder subjektiven Zeichen fur den kommenden Infekt aufwiesen

Der direkte Einfluss des Tabakrauches auf die Nasenschleimhaut an 4 Probanden ist als Pilotstudie anzusehen und erbrachte schon wegen der kleinen Zahl von Untersuchungen keine signifikanten Ergebnisse

Die Untersuchungsergebnisse uber die

Transportbeeinflussungen durch Xylometazolin sind jedoch als signifikant zu bezeichnen da die Versuche ohne Beeinflussung der Transportfunktion durchwegs eine deutlich hohere Transportgeschwindigkeit aufweisen Es ist mit dieser Messmethode nicht zu klaren ob es sich hier um einen Primareffekt oder um einen Sekundareffekt durch Verringerung des Atemwiderstandes und der durch Vasokonstriktion erfolgte Erniedrigung der Schleimhauttemperatur handelt Bei Untersuchung pharmakologischer Einflusse von Nasentropfen auf den Schleimhauttransport wurden fast ausschliesslich in vitro Studien durchgeführt Es ware zu empfehlen, dass bei weiteren Untersuchungen uber den direkten Effekt von Nasentropfen man auch Untersuchungen am Menschen durchführt

SUMMARY

The mucociliary transport of the human nasal mucosa was studied by using very small resin beads tagged with [5]Cr Several modifications of previous methods were introduced e g kind of nuclide particle size pH mode of application measuring technique and reduction of local irradiation Finally arrangements implying exact measurements of transport not only horizontally but also vertically or obliquely were obtained No mucociliary transport was demonstrated in five of nine subjects with a common cold 10 days before or one week after the investigation Xylometazolin as a nasal spray diminished the mucociliary transport significantly In addition the effects on mucociliary transport caused by homolateral or contralateral experimental nasal obstruction as well as by tobacco smoking were studied in healthy subjects Finally patients with various diseases pollen allergy in free intervals chronic rhinitis septal deviation or perforation and condition after laryngectomy were also investigated

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Anhang

SCHÄTZUNG DER BESTRAHLUNGSDOSIS BEI MESSUNG DES SCHLEIMHAUTTRANSPORTES MIT ^{51}Cr UND $^{99}Tc^m$ MARKIERTEN PARTIKELN

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Der erste Kandidat, welcher als Markierungs substanz zum Studium des Schleimhauttrans portes ausgewahlt wurde, war $^{99}Tc^m$-Sulfurkolloid, das gleichzeitig zum Studium der Leber in der Abteilung fur Nuklearmedizin herge stellt worden war Die Voruntersuchungen zeigten, dass das Kolloid einen guten Schleim hauttransport in der Nase des Kaninchens ge wahrleistete aber die Dosisschatzungen enthullten, dass durch diese radioaktive Trager substanz eine beachtlich hohe Bestrahlungs dosis auf die Flimmerepithelzellen in Form von Elektronenstrahlen, als Zerfallsprodukt von $^{99}Tc^m$, trifft

Die Untersuchungen am Menschen wurden daher mit jonenaustauschenden Harzpartikeln durchgefuhrt welche mit ^{51}Cr markiert wurden

Die Dosisberechnungen wurden an Hand ei Modells durchgefuhrt welches genau die beim Menschen vorhandene Situation simulierte und beruhte auf Elektronenzerfall und transportberechnungen welche durch das MIRD-Committee veroffentlicht worden sind (1969, 1971) Es wurde angenommen, dass die Aktivitat homogen in Form einer unendlich dunnen Schicht auf der Schleimhaut zu liegen kommt, der aktive Bezirk einen Durchmesser von 3 mm hat und dass dieser Bezirk in dieser Form und Ausdehnung wahrend des ganzen Transportes bestehen bleibt

Zwei Punkte im Innern der Schleimhaut wurden bei der Kalkulation betrachtet, ein Punkt 30 μm unter der radioaktiven Schichte, der zweite 70 μm tiefer Die Totalaktivitat von $^{99}Tc^m$ mit 3 μCi und von ^{51}Cr mit 30 μCi wurden jeweils angenommen Diese Aktivitaten ergeben annähernd dieselben Zählraten in der Gammakamera unter experimentellen Beding ungen Einzig die Elektronenstrahlung wurde in den Kalkulationen in Rechnung gesetzt wobei drei Situationen angenommen wurden

a) Die Aktivitat verbleibt unendlich lang an der Applikationsstelle liegen
b) Die Aktivitat bleibt an der Applikations stelle liegen und wird nach 20 min zur Gan ze entfernt
c) Die Aktivitat wird mit einer Geschwindig keit von 1 mm/min weitertransportiert

Schliesslich wird angenommen dass die Elektronenquelle von einem unendlichen Volumen Wasser umgeben ist

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Tabelle *Strahlendosis der Elektronen an zwei verschieden Tiefen der Schleimhaut*

Tiefe (μm)	Nukleid	Aktivität (μCi)	Dosis (rad) Kein Transport Keine Partikel entfernung	Kein Transport Entfernung nach 20 min	Transport geschw 1 mm/min
30	$^{99}Tc^m$	3	600	20	3
	^{51}Cr	30	900	0 3	0 04
100	$^{99}Tc^m$	3	200	9	1
	^{51}Cr	30	600	0 2	0 03

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BIPOLAR ELECTRIC STIMULATION TO ELICIT AN ISOLATED TENSOR TYMPANI REFLEX

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Abstract A bipolar electrode is described designed to obtain improved reflex responses from the tensor tympani muscle by electric stimulation of the under surface of the tongue d c generator, 7–9 V d c In comparison with the previous unipolar method of stimulation the responses under oscilloscopic analysis appear very constant and of greater size The latency in normal subjects is 117 msec

In a previous paper (Bosatra et al , 1975) the possibility was demonstrated of eliciting an isolated tensor tympani muscle reflex (t t m r) contraction by an electric stimulation of the tongue, which constituted an improved method in comparison with the air puff technique, etc

In the previous investigation, consistent responses have been obtained in normal individuals and in subjects affected by pathology of the tympano-ossicular system or of the nervous arch in its afferent portion (lingual nerve), intermediate portion (brain stem level) and efferent portion (motor branch of trigeminal nerve)

The stimulus, of 1–2 mA, was given through a positive and/or a negative electrode (28 mm^2) applied direct to the tongue and the circuit was closed by pressing one hand against a copper plate The responses recorded through an impedance meter and an oscilloscope consisted of positive or negative spikes at random, of small magnitude

Although the method was considered to be quite reliable, a further improvement has been achieved recently by applying the electric stimulus, obtained by a d c generator, to the under surface of the tongue through a bipolar electrode, with the following specifications diameter of each rod, 2 mm, distance between rods, 7 mm (Fig 1) In normal subjects the voltage required to obtain the reflex was 7–9 V d c

A small series of stimuli was given, of 100 msec duration, and with intervals of 2–3 sec, at random Prolongation of the stimuli did not improve the responses, which appeared as a positive spike, rarely a negative one, relative to the base line (Fig 2) The average latency of the reflex, mean value of 50 normal subjects, was 117 msec

A complete absence of response has been observed after 11–12 shocks (in some cases after only 3–4 shocks) and the contraction did not appear even when the lingual area of stimulation was changed, homo- or contralaterally (This phenomenon points toward an exhaustion of the effector muscle and not of the receptors or afferent nerve of the stimulated area)

A subsequent puff of air against the eye elicited complex responses, because of an associated contraction of the stapedius, and/or of the articular muscles Thus, these contractions could not be taken as a demonstration of the responsiveness of the tympanic [illegible]le itself

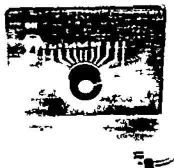


Fig 1

In conclusion, the bipolar electric stimulation of the tongue represents a very reliable method of eliciting t t m r contraction which is easily recorded as a very uniform and clear spike The avoidance of the elevated and variable body electric resistance from the hand to the plate represents an appreciable improvement of the method

ACKNOWLEDGEMENT

The Authors are indebted to Mr E Tulliach for technical advice and construction of the instruments

RÉSUME

Les AA decrivent l utilisation d une électrode bipolaire pour obtenir des reponses réflexes nettes du muscle tensor tympani par une stimulation électrique de la langue Ils emploient un génerateur DC de 7–9 V d c Par rapport à la méthode precedente de stimulation unipolaire, les réponses analysees à l oscilloscope sont bien plus constantes et importantes La latence dans les sujets normaux, est de 117 10 msec

ZUSAMMENFASSUNG

Die Verfasser beschreiben die Verwendung einer bipolaren Elektrode um eine sichtbare Reflexkontraktion des Musculus tensor tympani durch eine elektrische Reizung der Zunge zu erreichen Es wird ein Generator d c von 7–9 V d c verwendet Im Vergleich fruher angewendeter Methoden bezuglich der Unipolar Stimulation sind die Antworten—am Oszillographen analysiert—weit konstanter und großer Die Latenz betragt in normalen Fällen 117, 10 msec

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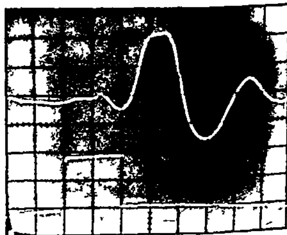


Fig 2

Acta Otolaryngol 83 393–400, 1977

EFFECTS OF PERILYMPHATIC PERFUSION WITH NEOMYCIN ON THE COCHLEAR MICROPHONIC POTENTIAL IN THE GUINEA PIG

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Abstract The effects of three concentrations of neomycin administered by a method of acute perilymphatic perfusion of the guinea pig cochlea on the cochlear microphonic potential (CM) at 4 kHz and 500 Hz are described A concentration-dependent reduction in CM occurred during the 60 minute perfusion period Neomycin at 10^{-4} M did not change the CM magnitude while at 10^{-3} and 10^{-2} M it caused 4 kHz (and 500 Hz) CM reductions which began within 24 (for both frequencies) minutes and 10 (and 12) minutes of drug application respectively CM reduction proceeded at a higher rate for greater neomycin concentration The perfusion technique the implication of the frequency indifference and the potential of the perfusion technique for inner ear biochemical analysis are discussed

The destructive effect of the aminoglycosidic antibiotic neomycin on the inner ear tissues and the resulting loss of cochlear AC potential (cochlear microphonic CM) after repeated daily administration of the drug has been reported by Hawkins (1952) Repeated doses of neomycin by injection result in a variable concentration of the drug in cochlear perilymph (Voldrich, 1965) The concentration is a function of several variables most importantly the availability and clearance of neomycin from the blood

One technique which provides the capability of holding a constant concentration of an ototoxic agent in perilymph is that of cochlear perilymphatic perfusion The basic method has been in use at least since the experiments of Tasaki & Fernandez in 1952 Much of the research using the perfusion technique has been directed at the study of metabolites and of ionic properties of perilymph or endolymph (Honrubia et al , 1965, Konishi et al 1966 1967, Konishi & Kelsey, 1968, Kuijpers, 1969, Prazma, 1969, Schnieder, 1970, Jung, 1971) While some of these studies used ototoxic agents (see also Schindler, 1973), the investigation of aminoglycoside ototoxicity by monitoring short term CM changes during perfusion of the cochlear perilymphatic space is a technique with great potential research value that is little used at this time CM changes that result from topical application of antibiotics to the fish lateral line organ have been reported by Wersall & Flock (1964)

It is the purpose of this paper to report the use of the perfusion technique and the answers thereby obtained to the following questions Can CM changes be observed during a relatively short (compared with chronic injection) 60 min acute perfusion of the cochlea with neomycin? Is the frequency effect of perceptual hearing loss which is progressive from high to low frequencies with chronic daily administration of neomycin also manifested in CM loss at high and low frequencies during acute perilymphatic perfusion with constant neomycin concentration?

This study was presented in part at the 89th meeting of the Acoustical Society of America in Austin Texas April 1975 It was supported by Public Health Service Program Project Grant 05785 and NS 11731

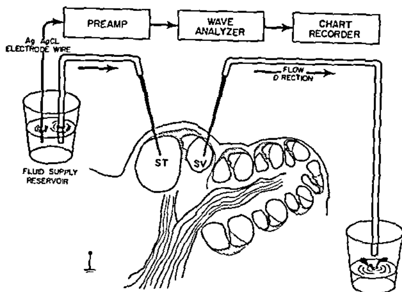


Fig 1 Schematic representation of the perfusion and recording system *ST* Scala tympani *SV* scala vestibuli

METHODS

More than 70 albino guinea pigs were used to develop the technique and obtain the data described below The animals were anesthetized with a urethane and diallylbarbituric acid (400 and 100 mg/ml respectively) anesthetic administered intraperitoneally at the dose of 0 8 ml anesthetic/kg body weight Artificial respiration was provided through a tracheal ... and the heart rate was monitored continuously during the experiment A conventional ventral surgical approach was used to expose the left auditory bulla which was opened, without disrupting the tympanic membrane or ossicular chain, to allow access to the cochlea The head was fastened in a head holder and a closed field sound delivery tube was connected to the ring of cartilage at the meatus which remains after removal of the left auricle

Using a hand held drill fashioned by sharpening a point onto a jeweler s screwdriver two holes (approx 0 1 mm ∅) were drilled into the first turn of the cochlea The first hole opened into the scala tympani of the basal turn while the second hole went into the scala vestibuli The position of these holes was the lowermost portion of the basal turn before the basal "hook ' begins to form approximately 2 5 mm from the round window

Two perfusion capillary assemblies were each fabricated by attaching a length of polyethylene tubing (Intramedic PE60) to a 5 cm length of glass tubing (0 8 mm OD) which had the other end drawn out to a fine tip by a microelectrode puller

A solution of artificial perilymph (derived from Rauch & Kostlin, 1964)[1] was used to prefill (from syringes) the capillary systems It was important that there be a complete, bubble free column of fluid in each capillary system

The size of a glass capillary was adjusted by breaking the tip until it was a close fit into a drilled hole and could be positioned and advanced into the hole using a micromanipulator It was not necessary that the opening be completely sealed by the capillary because cement (Durelon Carboxilate dental cement, Premier Dental Products) was then used to provide a leak free connection One perfusion capillary was cemented into each of the two holes drilled into the cochlea

After both capillaries were positioned cochlear flow was established by first disconnect

[1] Artificial perilymph had the following composition 130 mM NaCl 10 mM $NaHCO_3$ 4 mM KCl 1 mM $MgCl_2$ 1 5 mM $CaCl_2$ 1 mM Na H_2PO_4 20 mM HEPES pH 7 4 (if buffered)

ing the tubing from the filling syringes, placing the inlet tubing into the perfusate supply reservoir, then lowering the outlet tubing (scala vestibuli) below the level of the inlet (scala tympani) to cause syphon flow Perfusate enters the perilymphatic space of scala tympani, traverses the cochlear spiral, the helicotrema, and exits from the scala vestibuli This experimental apparatus is schematically shown in Fig 1

Perfusion rate was controlled by adjusting the differential height between the inlet and outlet tubing and was, of course, also a function of the fluid viscosity and the various flow resistances (primarily the glass capillary tips and the helicotrema) Various rates were used during the study ranging from 10 to 80 μl per minute A flow rate of approximately 20 μl/min was most commonly used

After flow was initiated, perfusion with artificial perilymph was continued in order to allow the CM, for constant sound intensity, to reach and maintain at a steady level for 15–30 min Animals were eliminated from the study if their CM did not reach steady state during the initial period of perfusion with artificial perilymph The contents of the supply reservoir were then exchanged for the experimental solution containing 10^{-2}, 10^{-3}, or 10^{-4} M neomycin B in artificial perilymph The rate of flow and the known volume of the inlet capillary system allowed determination of the time when the drug reached the cochlea

All perfusates were allowed to equilibrate to room temperature and to atmospheric gas concentration before and during perfusion The perfusate probably caused some steady state cooling of the cochlea and this would be a factor in the CM changes on initiation of perfusion To control the pH changes of artificial perilymph, caused by the release of CO_2 (from bicarbonate), the solutions used in more recent experiments were buffered to pH 7 4 with HEPES buffer

The perfusion technique described above is similar to that used by Schmieder (1974) and different from most others in that the perfusate is not allowed to collect in the bulla after exiting from the cochlea Rather, the perfusate is led away through an exit tubing system The inlet–outlet tubing system has the advantages of allowing precise control of the flow rate while permitting a stable CM, as there is no build up of extraneous fluid around the cochlea, the round window, or the recording electrode

Change in the magnitude of the CM signal (4 kHz or 4 kHz plus 500 Hz acoustic stimulation) in response to neomycin application, was the measured electrophysiological parameter A stimulus of 4 kHz was chosen because the site of maximal basilar membrane activity would be close to the recording electrode and close to the entry point of the drug into the cochlea Also, chronic drug experiments show progressive CM and hearing loss beginning with high frequencies (Carr et al, 1951, Hawkins, 1952, Ward & Fernandez, 1961, Farkashidy et al, 1963, Stebbins & Coombs, 1975) The 4 kHz CM signal was recorded, in most experiments, directly from the scala tympani perfusion capillary as if it were a micropipette electrode, by means of a silver wire placed into the supply reservoir In a few early experiments, before this recording technique was developed, the recording electrode (for 4 kHz) was a copper bead placed on the round window membrane Either recording method would give the same result but round window electrodes must be watched for fluid accumulation around the electrode which could shunt electrical signals

In all cases where 500 Hz was recorded CM was picked up by a copper bead electrode placed on the third turn of the cochlea The 500 Hz signal could just as well have been taken from the other electrode (for 4 kHz) but the bead placement was adopted early and maintained for reasons of consistency Reference for the single-ended signal recording was a silver wire placed in the neck muscles

Electrical signals were amplified 1000× (Princeton Applied Research CR-4 Preamplifiers) and measured on wave analysers (Hew

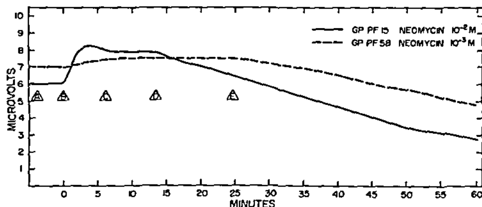


Fig 2 Examples of the changes in CM during 60 min perfusions with 10^{-2} and 10^{-3} *M neomycin* *A*, *Neomycin* placed in the supply reservoir, *B*, Neomycin enters the perilymphatic space of the cochlea *C* CM reaches a new steady level The level shift is caused by flow rate *changes and pH shifts*, *D* *CM begins to be depressed by* 10^{-2} M neomycin, *E*, CM begins to be depressed by 10^{-3} M neomycin

lett-Packard 302 A and Hewlett Packard 3590A/3591A) Signal level was recorded on a chart oscillograph (Grass Instruments Co , Model 7) Initial CM output from the cochlea was adjusted to a level between 5 and 10 μV RMS for each frequency

Some of the experimental cochleas were fixed by perilymphatic perfusion of Maximow solution and processed by the standard histo ' techniques of decalcification, celloidin bedding, and sectioning to allow observation of the possible mechanical damage to the membranous labyrinth which may have been produced by the perfusion technique Animals showing evidence of Reissner's membrane rupture were eliminated from the data analysis

Controls

Many control experiments were done in this study to evaluate the numerous variables which might affect CM level (other than neomycin) By far the most significant variable, which can influence the absolute magnitude of the CM, is the pressure differential which forces the perfusate through the perfusion system Actually, that portion of the total pressure which is across the cochlear partition is most important Greater pressure results in lower CM level, for a given stimulus intensity However, the CM will remain at a constant level for a given constant pressure and that pressure will result in a constant flow rate The flow rate is a function of pressure, fluid viscosity, and the collective resistances of the capillary tubing and the helicotrema of the cochlea Differential changes in the resistance parameters can act to reduce the pressure across the cochlea, causing an artifactual increase in CM when a higher viscosity perfusate reaches the cochlea from the inlet tubing CM shifts to 'new' steady state levels were produced by control perfusions of artificial perilymph of various viscosities produced by adding high molecular weight Dextran The possibility that traveling wave changes also occur has not been ruled out Aside from the pressure effect, this study, (as also that of Berndt et al , 1975), shows that flow alone has no effect on CM (0 to 80 μl/min) The flow rate of 20 μl/min used in the present study was sufficient to avoid significant drug dilution by natural perilymph turnover Cochlear perilymph volume (16 μl) is completely exchanged by natural turnover in approximately 20 min (Schnieder, 1974)

Another variable investigated was the effect of perfusate pH change on CM Unbuffered artificial perilymph tends to become basic with exposure to air Exchange of 'old perfusate

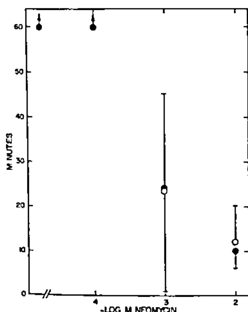


Fig 3 Mean delay times to start of CM decline at 4 kHz (O) and 500 Hz (●), concentrations Vertical range bars represent one standard deviation from the mean Control and 10^{-4} M neomycin caused no decline in CM by 60 min

with that of a freshly prepared sample can introduce a pH shift toward neutrality This pH shift was demonstrated to cause a corresponding increase in CM Jung (1971) has demonstrated the usefulness of HEPES buffer in artificial perilymph and in the present study control experiments showed that the buffer itself did not affect CM level during the control perfusion period

RESULTS

In Fig 2, examples of the effect of neomycin, at two concentrations, on the CM at 4 kHz are plotted for a 60 min perfusion The measured parameter is the length of time from the first arrival of neomycin in the perilymphatic space to a point judged to be the start of a steady decline in CM magnitude (labeled "D and E" for 10^{-2} M and 10^{-3} M concentrations respectively) Each of the sample curves exhibit an increase in CM which is coincident with the arrival of neomycin at the cochlea (time between "B" and "C") In a short time the CM settles to a new steady state value and it is from this new value that the measurements are taken

The early effect (between "B" and "C") is an artifact of the method The results of the formentioned control experiments performed to study the phenomenon, can be summarized as follows (*a*) Any manipulation, such as changing perfusate viscosity, which results in a change in the pressure differential across the cochlear partition will change the CM magnitude An increased pressure differential will result in smaller CM magnitude (in all cases scala tympani pressure was equal to or greater than scala vestibuli pressure) (*b*) Perfusate pH must be held constant for the duration of the experiment The use of a buffer in the artificial perilymph is therefore required because pH shifts can result in CM level shifts

Both of these factors, pressure and pH change, are potentially present when the experimental perfusate solution is substituted for the control perfusate

Fig 3 presents, for three concentrations of neomycin and control, the mean delay time, with standard deviation, to the first observed change in 4 kHz and 500 Hz CM Neomycin at 10^{-2} M produces a decline in CM which begins with a mean of 10 3 min (4 kHz, 13 ani-

Table I *Percentage of CM remaining after 60 minutes of neomycin perfusion at three concentrations and control and the mean delay time to beginning of CM reduction for these animal subsets*

Neomycin concentration	10^{-2} M	10^{-3} M	10^{-4} M	Control
Mean percentage CM (at 4 kHz) remaining at 60 minutes	22 %	68 %	100 %	100 %
S D	16	25		
n	7	13	4	9
Mean delay time for above animal groups (minutes)	9 6	29 5	>60	>60
S D	4 9	22 9		

Table II *From 16 fixed and histologically studied cochleas, data are grouped according to quality of CM and observable Reissner's membrane damage*

[illegible] mechanical damage

	No of Animals with intact Reissner's membrane	No of Animals with broken Reissner's membrane
No of Animals with stable and normal CM	10	0
No of Animals with unstable and low CM	1	5

mals) and 12 3 min (500 Hz, 12 animals) after application, and continues for the duration of the perfusion At a concentration of 10^{-3} M the mean delay to decline is 24 4 min (4 kHz, 14 mals) and 23 6 min (500 Hz, 11 animals) [illegible] the slope of CM loss with time being, in [illegible], less than that for 10^{-2} M [1] The lowest concentration of neomycin tested (10^{-4} M in 4 guinea pigs) did not produce a change in CM during the 60 min perfusion period as was the case for 6 control animals

Using only paired data, the delays obtained for both frequencies may be statistically compared A dependent Student's t test reveals that the latency times to CM decline of the two tested frequencies (4 kHz and 500 Hz) are not significantly different at either of the two effective neomycin concentrations (10^{-3} M, $N=11$, $T=0$ 11 10^{-2} M $N=10$ $T=0$ 57)

CM loss curves for the 4 kHz stimulus, were also analysed so as to determine the proportion of CM remaining at the termination of the perfusion period (Table I) Table I also lists the delay time statistics for the subset of experimental animals for which the CM remaining at 60 min could be obtained Using a straight line approximation, the slopes of the two loss functions may be calculated as −1 55 *and −1 05 for* 10^{-2} *and* 10^{-3} *M neomycin re*spectively

The cochleas from 16 perfusion experiments processed for histological examination showed an excellent correlation between quality of CM and observable mechanical damage from perfusion (see Table II) The most significant type of mechanical damage is a rupture of Reissner's membrane, caused primarily by drilling into the otic capsule or the placement of the perfusion capillaries

Based on the analysis of the histological data (Table II) it is possible to assess potential damage to the inner ear from the perfusion technique itself by observing the quality of the CM (i e 'normal starting magnitude and steady level) An ear with ruptured Reissner's membrane could potentially have a different time course of electrophysiological change because the drug may travel through altered diffusion pathways Table II is a summary of the histology of 15 ears which group primarily into the two classes where CM quality is good and histology shows no mechanical damage to the inner ears (10 ears) or CM quality is poor and inner ear damage is detectable (5 ears)

DISCUSSION

The effect of neomycin at concentrations 10^{-3} M or greater is to cause reduction in CM magnitude which is a function of the concentration The greater statistical variance seen for the 10^{-3} M latency suggests that this concentration may be a critical value for ototoxic action and therefore is a reasonable test concentration Greater neomycin concentration produces a relatively earlier change in CM and

[1] CM from one additional animal in the 4 kHz 10^{-3} M group and two additional animals in the 500 Hz 10^{-3} M group did not change during the test period It is probable that the CM would have decreased if sufficient time had been allowed These animals were not included in the data analysis

the reduction proceeds at a relatively faster rate The observed changes, taking place as they do within 60 min, are quite fast compared with the more gradual long term auditory, histological, and electrophysiological effects noted in studies using chronic daily injection of the antibiotic (Carr et al, 1951, Hawkins, 1952, Stebbins & Coombs, 1975) These data are probably not at variance, for several reasons Most importantly the 10^{-2} and 10^{-3} M concentrations of the ototoxic drug used in the present study are higher than that which reaches perilymph during the first days of daily injection Stupp (1970), measured a peak perilymphatic neomycin concentration of slightly more than 10^{-4} M within one hour after injection (100 mg/kg, sc) followed by a steady decline in concentration Daily injections result in a non monotonic build up of neomycin in perilymph (see Voldrich, 1965), while the perfusion technique holds concentration constant Nothing is yet known about the ability of mammalian auditory tissue to recover its function during the clearance phase of concentration change with daily dose, as has been noted for low concentrations of streptomycin topically applied to the fish lateral line organ (Wersall & Flock 1964)

It is clear, from an inspection of the data in Fig 3, that there are no significant differences between the delay times for 4 kHz and those for 500 Hz One might predict an earlier effect on the high frequency CM based on the studies in which neomycin was administered by chronic systemic injection For the progressive hearing losses reported in humans (Carr et al, 1951) and animals (Stebbins & Coombs, 1975), the higher audio frequencies seem to be affected earlier than the low frequencies

The results of the present study indicate that, if perilymphatic concentration of neomycin is held constant at a relatively high level CM generators in both the base and upper cochlear turns are equally vulnerable to the ototoxic agent

In order to explain the conflict between the hearing loss in chronic studies and the present CM data, with regard to frequency-specific effects, one could speculate that there are differences in routes of access and clearance of neomycin from the cochlea in the case of systemic drug application Such differences could be attributed to a differential (from base to apex) access of neomycin from the blood vessels of the stria into scala media and/or to differential turnover rates for perilymph or endolymph from the base to apex

CONCLUSIONS

The application of the perfusion technique to the study of neomycin ototoxicity, assessed by the CM changes, has been demonstrated here Clearly, the technique is well suited to the investigation of other aspects of ototoxicity (e g testing of other drugs, drug perfusion combined with various temporal periods of loud sound, possible recovery of function after perfusion) and of auditory physiology in general (e g changing perilymph viscosity for the study of cochlear mechanics)

Another important aspect of this method is that it can also be a tool for quantitative analysis of inner ear metabolism Radioactive precursors can be introduced into the cochlea without disturbing its function Tissues can then be dissected out and analysed quantitatively for radioactivity in selected metabolites Since CM can be monitored during the application of radioisotopes we have a procedure at hand with which to investigate biochemical reactions and CM under identical conditions An example is given in the accompanying paper (Stockhorst & Schacht, 1977) Short term reduction in CM magnitude, reported by the present study, suggests the cell membrane as a possible site of neomycin activity Changes of inner ear phospholipid metabolism first seen in chronic experiments (Orsulakova et al, 1976), are correlated with the above electrophysiological data The lipids under investigation in the accompanying paper have been linked to membrane permeability changes in excitable cells

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ZUSAMMENFASSUNG

Neomycin wurde mittels akuter perilymphatischer Perfusion in die Meerschweinchenschnecke eingebracht und die Wirkung dreier verschiedener Drogenkonzentrationen auf das cochleare Mikrophonpotential (CM) bei 4 kHz und 500 Hz wurde gemessen Wahrend sechzigminutiger Perfusion erniedrigte Neomycin das CM in Abhängigkeit von der Konzentration Keine Veranderung des CM wurde mit 10 ⁴ M Neomycin beobachtet aber 10 ³ M und 10 ² M riefen eine Reduzierung des CM bei 4 kHz (und 500 Hz) hervor die 24 (24) Minuten beziehungsweise 10 (12) Minuten nach Anwendung des Antibioti [illegible]

für biochemische Studien des Innenohres werden d skutiert

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RADIOACTIVE LABELING OF PHOSPHOLIPIDS AND PROTEINS BY COCHLEAR PERFUSION IN THE GUINEA PIG AND THE EFFECT OF NEOMYCIN

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Abstract Phospholipids and proteins of guinea pig stria vascularis spiral ligament and organ of Corti were radioactively labeled by perilymphatic perfusion with artificial perilymph containing [^{32}P]orthophosphate or radioactive amino acids Phospholipids were separated by thin layer chromatography, proteins by disc gel electrophoresis and quantitated by liquid scintillation counting The addition of 10^{-4}M to 10^{-2}M neomycin to the perfusion fluid resulted in a dose-dependent increase of tissue permeability to the radioactive precursors and a specific decrease in the ^{32}P incorporation into phosphatidylinositol diphosphate in stria vascularis and organ of Corti No effect of neomycin on protein labeling was observed using a double label approach with [^{3}H]methionine and [^{35}S] methionine *In vitro* low concentrations of neomycin led to the formation of a complex with polyphosphoinositides Much higher concentrations of the drug were needed for a comparable reaction with the acid mucopolysaccharide chondroitin sulfate A The implications of these findings for the mechanism of neomycin ototoxicity are discussed

The metabolism of proteins and lipids of inner ear tissues has been investigated mainly by histochemical or radioautographic procedures One of the most severe drawbacks of these methods is their inability to yield quantitative information about the individual proteins or metabolically important lipids present These and other limitations are well known and have been the subject of critical evaluation (Scarpelli 1970)

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Interference with cochlear protein metabolism has frequently been blamed in noise or drug-induced hearing loss Plester (1963) claimed radioautographic evidence for a decreased protein metabolism in the inner ear after chronic dihydrostreptomycin treatment Richrath & Kraus (1972) found reduced incorporation of intraperitoneally injected ^{3}H-DL-leucine into cochlear tissues and body organs (with the exception of liver) of the guinea pig after streptomycin injections The affected organs included brain, from which the antibiotic has been reported to be virtually absent after systemic application (Hawkins et al, 1950)

Lipids of the inner ear have previously been separated for qualitative studies (Scheibe et al, 1973), and we have recently shown that individual phospholipids of guinea pig inner ear tissues are amenable to quantitative radiochemical analysis (Schacht, 1974, Orsulakova et al, 1976) Phospholipids were chosen as objects of our studies because of their importance in cell membrane function (Michell, 1975) and their involvement in drug actions on membranes (Feinstein, 1964, Seeman, 1972)

The method of perilymphatic perfusion permits the introduction of controlled concentrations of both ototoxic drugs and biochemical precursors into the cochlea We

decided to investigate the influence of neomycin on the radioactive labeling of phospholipids and of proteins by this procedure These experiments were conducted parallel to electrophysiological studies under the same conditions and represent an attempt to correlate changes in cochlear microphonic potential with changes in cochlear metabolism

METHODS

The operation and perfuson of the guinea pig cochlea were carried out as described in the preceding paper (Nuttall et al , 1977) For radioactive labeling the "artificial perilymph" contained [^{32}P]orthophosphate ($^{32}P_i$) or radioactive amino acids Tissue dissection and lipid analysis have been described in detail elsewhere (Orsulakova et al , 1976, Schacht 1976*b*) In brief, following perfusion with the isotope, the cochlea was fixed with 10% neutral formaldehyde, tissues were dissected under the microscope and sampled as completely as possible from all turns The "organ of Corti" preparation contained both hair cells and supporting structures Tissues were extracted with an acidified solvent (chloroform/methanol/conc HCl, 3 2 0 05, by vol) after the addition of a guinea pig brain homogenate (1 mg protein) as a source of unlabeled carrier lipids For separation by thin-layer chromatography silica gel plates (Brinkman Silplate 60) were developed in chloroform/methanol/conc aqueous NH_3/H_2O (45 45 3 5 11, by vol) ^{32}P-labeled lipids were located by radioautography, scraped, and counted by liquid scintillation

For protein analysis, the tissues were collected, homogenized pelleted and washed twice in hot 5% trichloroacetic acid Further processing of the samples for disc gel electrophoresis in sodium dodecyl sulfate followed the procedure of Weber & Osborn (1969) Electrophoresis was carried out on 10% acrylamide gels Sample size was 100 µl At a constant current of 5 mA per gel and the positive electrode in the lower chamber, the marker dye bromphenolblue moved through the gel in approximately 6 hours Gels were fixed in 7% acetic acid/5% methanol, sliced, digested overnight at 60° in 30% H_2O_2, and counted by liquid scintillation

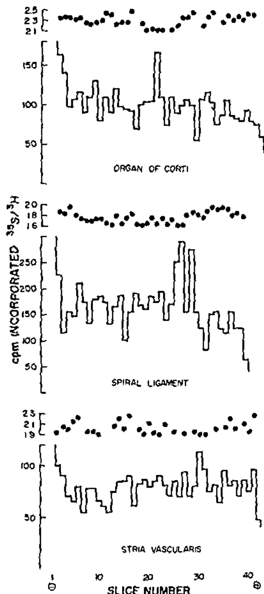


Fig 1 SDS-disc gel electrophoresis of labeled cochlear proteins Cochlear perfusions with 30 µCi [^{35}S]methionine/ml or 100 µCi [^{3}H]methionine/ml and electrophoresis of proteins on SDS-disc gels were performed as described in Methods Shaded area background cpm

For turbidity studies, polyphosphoinositides were prepared by chromatography on DEAE cellulose (Hendrickson & Ballou, 1964) from bovine brain Chondroitin sulfate A (purity>

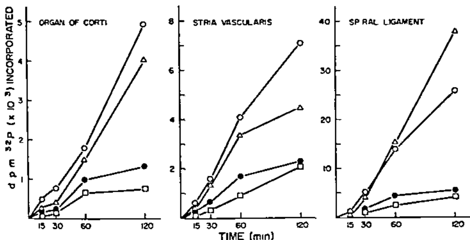


Fig 2 Time-course of ^{32}P incorporation into cochlear phospholipids Cochlear perfusions with 250 μCi $^{32}P_i$/ml were performed as described in Methods Each point represents the average of three experiments Δ–Δ phosphatidylinositol diphosphate, ○–○ phosphatidylinositol phosphate □–□ phosphatidic acid, ●–● phosphatidylinositol

95%) was a gift from Dr G W Jourdian of this University Dispersions of the lipids or the chondroitin sulfate in 20 mM Tris Cl (pH 7 4) were mixed with neomycin and turbidity was measured at 520 nm after reaching a stable absorbance (Feinstein, 1964)

Carrier free $^{32}P_i$ was purchased from New England Nuclear (Boston, MA), radioactive amino acids from Amersham/Searle (*L*-[methyl-^{3}H]methionine, 6 Ci/mmole, *L* [^{35}S]-methionine, 270 Ci/mmole) "Neomycin" in all experiments was Neomycin B sulfate from The Upjohn Co , Kalamazoo, Michigan

RESULTS

Cochlear proteins

Proteins from cochlear tissues can be resolved by gel electrophoresis For the investigation of protein labeling, amino acids of high specific radioactivity are needed This excludes ^{14}C-labeled amino acids but most tritiated amino acids are suitable Fig 1 shows the incorporation of another effective marker, [^{35}S]methionine, into cochlear proteins A number of radioactive peaks are clearly distinguishable, and results were essentially similar with [^{3}H]-methionine or with [^{3}H]leucine which was used in a number of experiments

Lipid labeling

The time course of ^{32}P incorporation into cochlear phospholipids shows (Fig 2) that the polyphosphoinositides (phosphatidylinositol phosphate and diphosphate) incorporate radioactivity more rapidly than other phospholipids

In spiral ligament there is an apparent short lag of incorporation which may reflect the slower penetration of $^{32}P_i$ from perilymph into the tissue After 60 and 120 min of continuous perfusion the labeling of phosphatidylinositol diphosphate exceeds that of phosphatidylinositol phosphate in spiral ligament, while the other tissues show the reversed pattern

Results in Fig 2 are given as average radioactivity incorporated into lipids If corrections are made for the amount of tissue and the radioactivity of soluble $^{32}P_i$ in each preparation (Table I) we find that stria vascularis shows the highest rate of lipid labeling, spiral ligament the lowest This supports other findings of high metabolic activity in stria vascularis (Thalmann et al , 1970)

The rate of ^{32}P-incorporation is remarkably consistent for different experiments If perfusion and dissection are rigorously controlled, different experiments can be compared on the basis of radioactivity in the lipids.

Table I *Incorporation of $^{32}P_i$ into inner ear phospholipids*

PhIP$_2$=Phosphatidylinositol diphosphate, PhIP–phosphatidylinositol phosphate PhA=phosphatidic acid PhI=phosphatidyl inositol The values for dpm ^{32}P lipid/dpm HCl soluble ^{32}P are given per mg protein

Tissue	Protein (μg)	HCl soluble ^{32}P (d p m)	^{32}P lipid/soluble ^{32}P			
			PhIP$_2$	PhIP	PhA	PhI
Stria vascularis	8	25 100	16 8	20 8	4 4	8 5
Organ of Corti	10	25 400	6 0	7 0	2 7	3 8
Spiral ligament	45	115 700	2 9	2 4	0 6	0 9

Perilymphatic perfusions with 250 μCi $^{32}P_i$/ml were carried out for 60 min as described in Methods Numbers are averages from three experiments protein values were determined from ten preparations

Normalizing to the tissue content of total ^{32}P, however, will eliminate possible differences between experiments in perfusion rate, dissection, etc The smallest variability between experiments is seen in the ratios of the ^{32}P-lipids This is understandable since this evaluation is independent of changes in the specific radioactivity of the precursor, sampling or analytical procedures For example, the average ratio of phosphatidylinositol diphosphate to phosphatidylinositol phosphate in four experiments was 0 67 for stria vascularis, 0 82 for organ of Corti, and 1 12 for spiral ligament a series of six experiments 4 months later , respective ratios were 0 72, 0 78, and 1 12 ıis data presentation was therefore selected for the results of neomycin treatment below Anomalous results should be expected to occur with technically imperfect perfusions, e g when Reissner s membrane is ruptured (see Nuttall et al , 1977) This was apparently observed in 2 out of 12 control animals Values for lipid labeling were subjected to Dixon s gap test (Dixon, 1950) and to Pearson & Stephens' (1964) test to check for normality of distribution and to identify out liers Data were eliminated when they were rejected by both procedures

Effects of neomycin

For the investigation of neomycin effects on amino acid incorporation into proteins a drug concentration of 10 $^{-3}$M was selected This concentration is sufficient to decrease the CM by 32% in 60 min (Nuttall et al 1977) The incorporation of [^{3}H]leucine into the total protein fraction (hot trichloroacetic acid insoluble material) remained essentially unchanged (Table II) For further investigation of possible effects on individual proteins, a double label approach combined with disc gel electrophoresis was employed in order to compare labeling of proteins in normal and drug treated animals Control animals received perfusions with [^{35}S]methionine, experimental animals with [^{3}H]methionine and 10^{-3}M neomycin Tissues from both groups of animals were collected and processed together This method eliminates artefactual differences that might arise during sample treatment, electrophoresis, slicing or counting The ratio of ^{35}S/^{3}H was finally determined in each slice of the electrophoresis gels If neomycin affects the labeling of a protein then this protein will show a ^{35}S/^{3}H-ratio that is different from the others These ratios are presented in Fig 1 for all peaks in which ^{3}H-labeling was at least twice

Table II *Effect of neomycin on incorporation of ^{3}H-leucine into protein*

[illegible]

acid insoluble material

Tissue	Controls cpm ^{3}H incorp	10 $^{-3}$M neomycin
Stria vascularis	367±88	350± 67
Organ of Corti	538±232	470±154
Spiral ligament	1 148±427	1 297±286

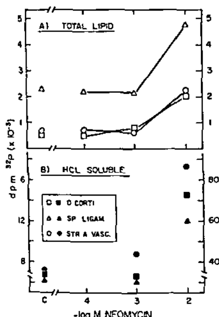


Fig 3 Effect of neomycin on $^{32}P_i$ and total ^{32}P lipids in cochlear tissues Cochlear perfusions with 250 μCi $^{32}P_i$/ml were performed as described in Methods with varying concentrations of neomycin Points are averages of four experiments Numbers on right ordinate are scale for spiral ligament *C*=control perfusions without drug

the background value Lower counts were considered unreliable Clearly, there is no evidence for a neomycin effect on the amino acid incorporation into any protein The deviations from the mean ratio are random and lie all within ±10% of this mean The experiment presented is representative of four different double label studies

Preliminary experiments had indicated changes in lipid labeling by neomycin Therefore, all concentrations of neomycin that had been tested in the electrophysiological study Nuttall et al , 1977) were used in these experiments The addition of neomycin to the perfusion medium produces concentration dependent changes in tissue radioactivity as well as in the pattern of lipid labeling At 10^{-2}M neomycin there is a dramatic increase in the amount of $^{32}P_i$ entering the tissues and consequently in the total amount of ^{32}P in lipids (Fig 3) When the incorporation into individual lipids was analysed by thin layer chromatography changes were found at 10^{-3}M and 10^{-2}M neomycin in the labeling of phosphatidylinositol diphosphate This decrease is shown in Fig 4 relative to phosphatidylinositol phosphate Both at 10^{-2}M and at 10^{-3}M antibiotic concentration there is a significant ($p<0.01$) drop in the polyphosphoinositide ratio in stria vascularis and organ of Corti Labeling in the presence of 10^{-4}M neomycin does not differ from the controls Although a similar trend is evident in spiral ligament, the differences are not statistically significant

In vitro effects of neomycin

A direct action of neomycin on polyphosphoinositides was studied *in vitro* An aqueous dispersion of a lipid fraction (80% phosphatidylinositol diphosphate, 20% phosphatidylinositol phosphate) was titrated with neomycin and complex formation was determined by measuring absorbance at 520 nm (Table III) Complexes between acid mucopolysaccharides and kanamycin had been described pre-

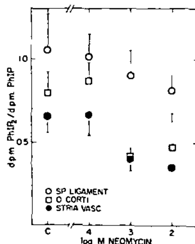


Fig 4 Effect of neomycin on ^{32}P incorporation into phosphatidylinositol phosphate and diphosphate Cochlear perfusions with 250 μCi $^{32}P_i$/ml were performed as described in Methods Points are averages of four to nine experiments each with S D indicated Significance of differences (One way ANOVA) 10^{-3}M neomycin and 10^{-2}M neomycin differ from control in stria vascularis and organ of Corti $p<0.01$ Other differences not significant *C*=control perfusions without drug PhIP PhIP₂ phosphatidylinositol phosphate diphosphate

Table III *Reaction of neomycin with polyphosphoinositides and chondroitin sulfate A in vitro*

A suspension of polyphosphoinositides (0 14 mM lipid 80% phosphatidylinositol diphosphate 20% phosphatidylinositol phosphate) in 20 mM Tris chloride (pH 7 4) was titrated with a 10 mM solution of neomycin Chondroitin sulfate A also in Tris buffer was 0 1 mM or 1 0 mM with respect to repeating units (approximately 0 05 and 0 5 mg/ml respectively)

Neomycin added (μM)	Polyphosphoinositide 0 14 mM (A_{520})	Chondroitin S 0 1 mM (A_{520})	Chondroitin S 1 0 mM (A_{520})
15	0 04		
25	0 15		
30	0 40		
40	1 36		
200	–	0 00	0 00
250	–	0 04	0 06
500	–	0 15	0 96
1 000	–	0 25	1 76

viously (Saito & Daly, 1971) and we compared the affinity of neomycin for polyphosphoinositides to its affinity for the mucopolysaccharide chondroitin sulfate A It is apparent that con lower drug concentrations lead to formation of complexes with the poly phosphoinositides

DISCUSSION

Perilymphatic perfusion is an efficient way of introducing large amounts of radioactive materials into the cochlea The analytical limits are set by the specific radioactivity of the precursor and the rate of its metabolism We have demonstrated here just two applications of this procedure for quantitative analysis of cochlear metabolism ^{32}P incorporation into phospholipids and amino acid incorporation into proteins Other metabolites such as sulfated or glycosylated polymers or RNA should be equally accessible for detailed studies There are two obvious advantages to the perfusion method First, the level of radioactivity that can be reached in the cochlear tissues is high enough to permit quantitative measurements of individual lipids or proteins from even a single cochlea Secondly, the potential of simultaneous or parallel recording of cochlear microphonic potentials makes this the procedure of choice for comparative studies of metabolism and function in the inner ear

The apparent lack of an effect of neomycin on the amino acid incorporation into protein during perilymphatic perfusion is not surprising Although protein synthesis has been invoked as a site of ototoxic action of the aminoglycosides, there seems to be little basis for this assumption Aminoglycosides exert their antibacterial actions by binding to the 30S *ribosomal subunit (Pestka, 1971) which does* not exist in this form in the mammalian cell Experimental evidence has shown that these drugs may have some effects on mammalian protein synthesis but that there are significant differences from the bacterial systems (Beard et al , 1969) Moreover, the positively charged antibiotics do not readily penetrate the cell membrane (Robson & Sullivan 1963) and quick responses of the microphonic potential to aminoglycosides—as shown by Wersall & Flock (1964) for the lateral line organ and by Nuttall et al (1977) in the guinea pig cochlea—suggest interference with bioelectric events at the cell membrane

Phospholipids have been repeatedly suggested as mediating such events in excitable tissues (Michell, 1975) Moreover, they are known to be receptor sites for a variety of drugs (Seeman, 1972 Feinstein 1964) The polyphosphoinositides have attracted particular attention because of their rapid turnover in excitable and in secretory tissues We find a rapid labeling of these lipids in inner ear tissues Radioactive orthophosphate perfused through the perilymphatic spaces is converted in the tissues to [γ-^{32}P] ATP and enters the polyphosphoinositides by two sequential kinase reactions

(I) Phosphatidylinositol+[γ-^{32}P] ATP
→ [^{32}P] phosphatidyl nos tol phosphate+ADP
(II) Phosphatidylinositol phosphate+[γ-^{32}P] ADP
→ [^{32}P] phosphatidylinos tol diphosphate+ ADP

This incorporation of ^{32}P into the monoesterified positions occurs considerably faster than the incorporation via *de novo* synthesis into other phospholipids. This finding is similar to our previous results (Orsulakova et al. 1976) which we had obtained by introducing the radioactive perilymph into the cochlea without continuous perfusion. In the present study we accomplish a much higher level of labeling and smaller variability between experiments.

The presence of neomycin in the perfusion fluid apparently interferes with the reaction sequence outlined above. The labeling of phosphatidylinositol diphosphate is decreased in the preparations of organ of Corti and stria vascularis, whereas lipid labeling in spiral ligament remains largely unaffected. This differential susceptibility of the inner ear tissues may reflect differences in neomycin penetration during the perfusion time. It is interesting to note, however, that we observed the same pattern after chronic systemic application of the drug (Orsulakova et al. 1976). Organ of Corti and stria vascularis are usually considered the primary sites of neomycin damage (Hawkins 1970).

As far as the molecular mechanism is concerned, an inhibition of reaction II could explain the results. However, we have presented evidence (Schacht 1976*b*, Lodhi et al. 1976) that neomycin can form a ionic complex with the polyphosphoinositides. Phosphatidylinositol phosphate in such a complex would not be a substrate for phosphorylation to phosphatidylinositol diphosphate (reaction II). As a result, labeling of the latter will be decreased.

Complexes of the basic aminoglycoside antibiotics with polyanions have been demonstrated with synthetic polymers *in vitro* (Cohen 1947, Mora et al. 1959) and the binding of these antibiotics to acid mucopolysaccharides has been discussed as a mechanism of their ototoxicity (Musebeck 1963, Saito & Daly 1971). It is obvious that any polyanion in the cell membrane can bind the aminoglycosides. The question therefore arises which of the potential receptors is primarily involved in the physiological action of neomycin? Although no direct evidence is at hand, the consideration of three points may help in the discussion: the affinity of suggested receptors for the drug, the metabolic consequences expected from the interaction, and the correlation between metabolic changes and physiological effects.

Drug/receptor interactions can be measured *in vitro*. Complex formation is associated with the formation of larger particles and an increase in the turbidity of the solution. This has been demonstrated for drug/lipid interactions (Feinstein 1964) as well as for kanamycin and mucopolysaccharides (Saito & Daly 1971). The direct comparison of polyphosphoinositides with the acid mucopolysaccharide chondroitin sulfate A clearly demonstrates that the lipid/drug complex is formed at neomycin concentrations ten to twenty times lower than those required for a comparable reaction with chondroitin sulfate A.

Secondly, an inhibition of polyphosphoinositide turnover should disturb function and integrity of the afflicted cells as we have discussed in detail elsewhere (Schacht 1976*a*). The polyphosphoinositides are considered typical constitutents of nervous or secretory tissues. Although their function is not firmly established, there is strong evidence that points to their involvement in the regulation of membrane permeability, possibly by their capacity to bind calcium (Michell 1975). Neomycin has been shown to displace calcium from inner ear tissues (Orsulakova et al. 1976) as well as from polyphosphoinositides (Lodhi et al. 1976). An action of neomycin on these lipids would then lead to disturbances of the membrane calcium equilibrium and of membrane integrity. In accord with the latter, we find in this study a pronounced effect of the drug on the penetration of $^{32}P_i$ into the tissues. This demonstrates that experimental approaches to neomycin toxicity which only measure changes of reaction products and disregard precursor concentrations will lead to misinter-

pretations It should also be considered that a membrane–antibiotic interaction might lead to secondary effects such as the inhibition of membrane associated enzymes

Lastly, but most importantly, we have to consider the close correlation between the biochemical changes induced by neomycin and the decrease of cochlear microphonic potential recorded under the same conditions (Nuttall et al, 1977) The similarity of the dose–response curves is strongly suggestive evidence for a link between the two events Thus, an interference by neomycin with polyphosphoinositide metabolism may well be the biochemical mechanism underlying the decrease in cochlear microphonic potential We must, however, leave the way open for other interpretations, since it would be premature to conclude a causal relation between the two observations

ZUSAMMENFASSUNG

Phospholipide und Proteine von Stria vascularis Ligamentum spirale und Cortischem Organ des Meerschweinchens wurden radioaktiv markiert mittels perilymphatischer Perfusion mit kunstlicher Perilymphe die [^{32}P] Orthophosphat oder radioaktive Aminosauren enthielt ... de wurden dunnschichtchromatographisch ... Proteine durch Polyacrylamid Gel Elektrophorese Radioaktivitat wurde im Flussigkeitszahler gemessen Die Zugabe von Neomycin (10^{-4} M bis 10^{-2} M) zur ...
ha...
den
^{32}P ...
vascularis und Cortischem Organ Doppelmarkierungsver suche mit [^{3}H]Methionin und [^{35}S]Methionin zeigten ...
... phosphoinositiden wahrend weit hohere Konzentrationen ...
Ototoxizitat des Neomycins wird diskutiert

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BEHAVIORAL AUDITORY FUNCTION AFTER TRANSECTION OF CROSSED OLIVO COCHLEAR BUNDLE IN THE CAT

III *Further Study of Ambient Light Intensity Discrimination under Intense Noise*

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Abstract This report describes the results in cats of a visual auditory dual modal experiment after translateral olivo-cochlear bundle ablation at the floor of fourth ventricle Subjects were behaviorally conditioned (avoidance) to respond in a shuttle box to show that they detected changes in ambient light intensity during the existence of intense background noise With the given experimental paradigm no noticeable difference was found between the response of the experimental animals and those of animals that underwent sham operation

Neurophysiological studies (Galambos, 1956, 1960, Fex, 1962) on the crossed olivo-cochlear bundle (COCB) have shown that that system's function may be inhibitory Later, Pfaltz (1969) reported that he found no function of the COCB in the guinea pig under physiological stimulus conditions Irvine & Webster (1972), measuring cochlear microphonics and auditory nerve action potentials in unanesthetized cats stated that the olivo-cochlear system does not have a peripheral gating mechanism

Many behavioral conditioning studies have been done involving the olivo cochlear system Dewson (1968) using 4 rhesus monkeys tested the effect on discrimination of human vowel speech sounds in the presence of noise In his study the COCB was cut in the midline and, in addition, the cortex was ablated in 3 of the 4 monkeys Capps & Ades (1967, 1968) reported a deficit in frequency discrimination performance at 1 000 and 4 000 Hz in squirrel monkeys after focused ultrasonic irradiation or surgical interruption of the COCB Trahiotis & Elliot (1970) reported that in cats, the extent of masking effect at 1 000 Hz and 2 000 Hz was slightly increased after COCB midline section, although the shift was not statistically significant We have also found, in cats that severing the COCB does not affect the behavioral measurement of pure tone thresholds and the perceptual signal to-noise ratio (Igarashi et al , 1972)

Guzman Flores & Alcaraz (1963) indicated that the olivo cochlear bundle lesion abolished the attenuation of cochlear evoked potentials while the subject was attentive to a visual stimulus Subsequently, we undertook a similar study, training cats to detect the occurrence of a visual signal in the presence of intense background white noise The visual task was the detection of a dim light signal presented through a small circular hole (1 3 cm in diameter) in a black box installed in front of the cat s rotating cage In contrast to the

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sham-operated cats, animals with midline COCB lesions showed difficulty in detecting the light signal in the presence of the intense noise (Igarashi et al , 1974) Because this task required the cat to watch vigilantly a relatively small area by orienting its head and neck toward the box, the COCB lesion could have a non specific effect on the animal s behavioral responses

Here we describe the result of a subsequent visual auditory experiment in which we used a shuttle box to condition cats to detect changes in ambient light intensity In this study, we attempted to simplify the cat's task by eliminating the need for maintaining any specific directional orientation to solve the task

SUBJECTS

A total of 10 adult cats were used in the present experiment They were randomly selected on the basis of general good health and temperament, clean external ear canals, and normal appearing tympanic membranes

APPARATUS

Each cat was trained with a shock avoidance procedure in a two-way Plexiglas shuttle box Auditory stimuli were presented from Lansing LE 85 speakers mounted 48 inches above the center of each compartment The speakers and shuttle box were inside an IAC Model 1202 sound treated chamber whose ambient noise level was about 35 dB re 0 0002 dyne/cm^2 The noise stimuli were produced by a Grason Stadler Model 455 C noise generator having a 0 8 kHz to 20 kHz band limited output The filtered output of the noise generator was routed to a Grason Stadler 829 E electronic switch

The light stimuli were produced by reducing the intensity of the ambient illumination level in the test chamber The ambient light originated from four 200 watt incandescent light bulbs mounted behind a translucent glass screen in the ceiling of the IAC chamber, 72 inches above the center of the test cage As measured from a point 15 inches below the light source, the ambient light level was about 180 foot-candles The stimulus light intensities of test situations I, II, III, and IV were 169 0 170 4, 171 7, and 173 1 foot-candles (with an average amperage at the light bulbs of 2 46, 2 48, 2 50, and 2 52 respectively) The test situation V with 174 5 foot-candles, was found to be too difficult for cats to discriminate from the neutral 180 foot-candles level, hence, we excluded those unreliable results from our study

A Grason Stadler 4711 interval timer controlled the onset and offset of the filtered noise and simultaneously controlled the closure of a relay that supplied power to the incandescent bulbs by means of a Triac a c power supply The noise pulses had a duration of 1 sec, with 1-sec interpulse intervals The rise–decay time of the noise pulses was 25 msec The onset of the light intensity decrement was delayed 25 msec relative to the onset of the noise, but was turned off simultaneously with the noise offset

PROCEDURE

Preoperative training and testing

During the first 3 days the cats were acclimatized to the test environment Each cat was allowed to explore the test cage for 25–30 minutes each day without any conditioning stimuli present Next each cat was trained to cross to the opposite compartment to avoid shock when go signals—10 sec periods of simultaneous noise (60 dB re 0 0002 dyne/cm^2)—were presented At that stage of the experiment the change in ambient light was about 11 foot candles (for example, from 180 to 169 foot candles) If the cat had not started to move to the opposite compartment at the end of 5 consecutive light noise pulses, mild electrical shock (manually controllable) was delivered to the cat's paws through the grid floor of the training apparatus Cats received 10 trials per

day with randomized intertrial intervals (ITI) ranging from 30 to 90 sec (mean, 60 sec) The criterion for completion of the initial training stage was 90% correct responses or better for 5 of 6 consecutive days

Next, we tried to determine what cues the cats used to solve the original task Over a 10 day period, each cat received 12 trials daily, 10 of which were retraining trials with paired light and noise stimuli, one in which only the light stimulus was presented and one in which only noise was presented No shock was given for failure to respond to these test trials

The final step before surgery was to measure each cat's detection threshold (50% correct response level) for the occurrence of noise pulses We used a modified method of descending limits to determine the threshold (Igarashi et al , 1972) By the threshold measurements, we were able to ensure that, in assigning cats to the sham and bona fide surgical groups, the two groups were about equal in terms of individual hearing sensitivity We also attempted to balance the two groups in terms of individual differences for ease of learning the original discrimination and for performance on the single, light or noise, test trials

Surgery

In operating on each of the animals, general anesthesia was induced by means of intraperitoneal injection of sodium pentobarbital, 30 mg/kg An occipital midline incision exposed the bony calvarium The occipital bone was removed with rongeurs in a piecemeal fashion to expose the posterior portion of the cerebellum and the brain stem The intention was made to make the bony opening large enough to avoid any postoperative side effect from edema The cerebellum mostly the area of the uvula and nodulus was gently elevated with a non rigid malleable retractor By using a fine pick the COCB was cut in the midline of the floor of the fourth ventricle at the level of the facial genu The midline incision was intentionally extended both rostrally and caudally beyond the edge of the facial genu During this surgery care was taken not to retract the cerebellum too far or for too long a time An identical surgical procedure was done on all sham controls, except that the COCB was not sectioned

Postoperative training and testing

The first two stages of postoperative testing were identical with the first two preoperative stages

Subsequent postoperative testing was designed to ascertain whether the presence of continuous noise of different intensity levels has a distractive effect on the cats' abilities to detect changes in ambient light levels To test for such effect, we first had to retrain the cats to respond to changes in light levels rather than to noise pulses The retraining was accomplished by gradually attenuating the intensity level of the noise pulses over successive days and trials while maintaining the size of the change in ambient light at the prior level of 11 foot candles This procedure was continued until each cat achieved a minimum of 90% correct responses for two consecutive days with the noise generator turned off

Next, we attempted to determine each cat's approximate threshold for detecting changes in the ambient light intensity levels Over days, the amount of change in the ambient light level was decreased in steps of about 2 foot candles until a level was reached at which the cats performance level dropped to 50% correct responses or below We then selected four levels of light changes (from 'easy to 'hard , graded I–IV) for use in subsequent testing

Next we attempted to measure the possible distracting effect of different intensity levels of continuous noise application on the easy to hard ' light intensity discriminations The different noise intensities were presented in random order The levels used were 40 60 80, 90, and 100 dB re 0 0002 dyne/cm² During the testing, the order of presentation of the four light level conditions were from easy

Table I *Corrected positive response rate*

Noise in dB re 0 0002 dyne/cm²	Light intensity I		II		III		IV		$\bar{X}$	
	X	S	X	S	X	S	X	S	X	S
40	0 71	0 68	0 71	0 68	0 77	0 68	0 49	0 42	0 67	0 62
60	0 88	0 69	0 72	0 79	0 73	0 71	0 54	0.56	0 72	0 69
80	0 87	0 65	0 75	0 62	0 60	0 65	0 52	0 41	0 69	0.58
90	0 75	0 84	0 74	0 72	0 66	0 63	0 48	0 34	0 66	0 63
100	0 77	0 68	0 72	0 73	0 59	0 64	0 48	0 40	0 64	0 61
$\bar{X}$	0 80	0 71	0 73	0 71	0 67	0 66	0.50	0 43	0 68	0 63

to hard, back to easy, then again to hard, etc In both procedures the noise intensity was switched to the next test level immediately after the end of each 10-sec trial period During all phases of the present experiment the rate of spontaneous crossing between trials was recorded and subsequently used to correct for the percentage of correct responses

When the behavioral testing was completed, we obtained specimens for morphological study After perfusing the inner ear with 2% osmium tetroxide solution (Millonig), we removed the cochlea and embedded it according to the standard Epon procedure Ultrathin sections were studied by means of an electron microscope The depth and rostrocaudal extent of surgical midline lesion was light-microscopically studied in the serial cross sections of the brain stem, which were stained in cresylecht violet

RESULTS

The results of pre- and post-operative tests with combined light and noise stimuli were identical in all cats (solving task on basis of noise)

Comparing the corrected positive response rates, we found no remarkable difference between the midline sectioned group (X) and the group undergoing sham operation (S) (Table I) When the light intensity discrimination became more difficult (I–IV) the corrected positive response rate declined in both the experimental group and the sham group We found that the different noise intensity levels affected the performance of both experimental and sham groups in the same way, even in the sessions with the "hard" light intensity discrimination

The corrected positive response rate showed a clear decline, regardless of the noise intensity level, when the light intensity discrimination level reached the level of 173 1 foot-candles (situation IV) The task was performable At the level of 174 5 foot-candles (situation V) the task was extremely difficult Therefore, the control on the light intensity variable in the present experiment had certain limitations

When we compared the average values of spontaneous crossings of 5 experimental subjects with those of 5 shams at each different level of task difficulty, we noted that, in 24 pairs out of 25, the rate of spontaneous crossings shown by experimental animals was greater than, or equal to, those of the sham subjects (Table II) The difference was significant at the 0 05 level (Mann-Whitney U test) This increase of spontaneous crossing was dependent on both the light discrimination task difficulty and on noise level Insofar as the difference between these two groups was the presence or absence of a midline section of the brainstem, the difference in spontaneous crossing rate could be partly reflecting the nonspecific effect due to the midline lesion itself

Table II *Rate of spontaneous crossing*

Noise in dB re 0 0002 dyne/cm²	Light intensity									
	I		II		III		IV		$\bar{X}$	
	X	S	X	S	X	S	X	S	X	S
40	0 10	0 06	0 09	0 08	0 12	0 11	0 15	0 14	0 12	0 10
60	0 08	0 08	0 13	0 08	0 13	0 10	0 17	0 15	0 13	0 10
80	0 12	0 16	0 15	0 12	0 21	0 14	0 19	0 19	0 17	0 15
90	0 23	0 15	0 19	0 13	0 21	0 18	0 22	0 18	0 21	0 16
100	0 15	0 12	0 18	0 13	0 25	0 18	0 28	0 22	0 22	0 16
$\bar{X}$	0 14	0 11	0 15	0 11	0 18	0 14	0 20	0 18	0 17	0 13

All brain stems and cerebella were examined light-microscopically No morphological alteration was found in the sham subjects In the experimental subjects, the midline sections had been placed appropriately and were extended about 1 0–1 5 mm rostrally and about 2 0 mm caudally beyond the level of the VIIth nerve genu Because of the long postoperative period before histology specimens were acquired (about 5 to 7 months), microscopic evaluation of midline lesions was difficult Furthermore, electron microscopic investigation of the organ of Corti in the ex- imental subjects confirmed that many ef- nt nerve endings to the outer hair cells disappeared Therefore, the results of both procedures confirmed the accurate placement of the COCB section

DISCUSSION

One must understand different experimental paradigms to compare results from our present behavioral study with those from our previous light–noise experiment (Igarashi et al , 1974) First, the task difficulty level of attentiveness, and training procedure differed In our previous study a cat rotating cage was used and the task involved the detection of a dim light signal (on–off) that was delivered through a small circular opening The prerequisite to this task was that the cat had to maintain a continuous direction-oriented vigilance to watch the region of the black box

Oatman (1971) had used a dual modal experimental paradigm Click-evoked potentials were recorded in unanesthetized cats having chronically implanted electrodes in the auditory cortex, cochlear nucleus, and round window The mean peak-to-peak amplitudes of averaged click-evoked (continuous free field background) responses all along the auditory pathway were clearly smaller during attention to the visual discrimination stimuli Oatman speculated that this selection process involved two systems the reticular feedback system and the olivo cochlear system His visual stimuli were concentric rings (mounted on one wall) presented successively for discrimination Cats had to respond to the onset of the small inner ring to receive food reward Thus, Oatman's cats were required to have a good vigilance level to maintain an orientation somewhat similar to that in our first experiment

In our present study, a detection of change in ambient light intensity was required of the subject Because the walls and ceiling of the double-grill box were constructed of clear Plexiglas, the cat was not required to watch any particular area or spot to detect the change in ambient light intensity The difference in requirement of a different concentrate level due to different task difficulty could be an important factor as far as the function of corticofugal connection to the olivo-cochlear system is concerned Even by merely observing the cats, the difference in their attentiveness was

distinguishable between two of our experiments By comparing behavioral training procedures, we used more gradual training and testing procedures with fewer trials per day for more trial days, in our present study

After midline COCB operations, some cats showed ataxia A 2-week postoperative recovery period generally resulted in sufficient recovery However, individual differences in ataxic condition (which tend to be worse in cats with COCB lesions) and partial ocular dyscoordination (which may result from an injury to the medial longitudinal fasciculus) might have produced differences in our previous experiment, by requiring that cats had to conduct a visual orienting response Because in our present experiment, cats were not required to watch any specific area the above described side effects should not influence the cat's discrimination performance

Hubel and others, in 1959 while recording responses of auditory cortex units in restrained and unanesthetized cats, found a population of cells that was responsive only when the subject was paying vigilant attention to the tone source (by looking toward it) That finding might account for some difference in our two light–noise study paradigms under the assumption that the cortico fugal influence is connected to the olivo-cochlear system function

Our series of functional experiments with COCB section in cats was initiated based upon the report that the majority of the olivo-cochlear bundle originates translaterally (Rasmussen, 1960, Konigsmark, 1973) However Warr s recent study (1975) on horseradish peroxidase in kittens showed that about 60% of the neurons were located on the side ipsilateral to the injection If Warr's results are substantiated, only limited effect may be expected by severing the COCB

RESUME

Ce rapport decrit les résultats fournis par une experience couplee audio-visuelle effectuee sur des chats après l ablation translatérale du faisceau olivo-cochleaire sur le fond inferieur du quatrième ventricule Les animaux ayant été conditionnes dans leur comportement (reaction d évitement) afin de reagir dans une boite navette et de montrer qu ils etaient capables de détecter des changements dans l'intensité de la lumiere ambiante pendant l existence d un bruit de fond intense Avec les conditions experimentales ainsi établies il n a pas été possible de detecter de différence remarquable entre les reponses des animaux qui subirent l ablation et ceux qui subirent l operation simulée

ZUSAMMENFASSUNG

Dieser Bericht beschreibt audio visuelle Versuchsergebnisse die mit Katzen auf zweierlei Arten unternommen wurden und zwar nach einer translateralen (von einer Seite zur andern) Sektion des OCB (Olivo-Cochlea Bundel) auf dem Boden des vierten Ventrikels Die Katzen wurden ihrem Verhalten nach so vorbereitet (durch Vermeidung) daß sie in einem Schleuderkasten erkennen ließen daß sie Veranderungen in der allerseitigen Lichtstärke bemerkten wahrend des Vorhandenseins von intensivem Hintergrundlarm Das gleiche Musterbeispiel wurde mit Katzen unternommen die nur eine Scheinoperation erhalten hatten Es wurde jedoch kein bemerkenswerter Unterschied festgestellt zwischen der Reaktion der Versuchskatzen und der zum Schein operierten Katzen

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TEMPORAL INTEGRATION OF ACOUSTIC ENERGY

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Abstract Perception of sound shows an increase in loudness related to the duration of the acoustic stimulus This phenomenon has been studied at threshold (temporal integration) in normal and impaired ears Normal ears showed an improved threshold with impulse durations up to 200 msec In cochlear hearing loss regardless of pathology the threshold improvement was reduced A method to evaluate these changes has been designed (Brief Tone Audiometry) When using this method a quantitative comparison showed the same reduction in temporal integration in ears with hearing loss regardless of pathology By using a loudness balance test the increase in loudness related to prolongation of the stimulus was investigated (Loudness Summation) Loudness growth was measured at various intensities and the results from normal and hearing-impaired ears were compared At a given sound intensity both normal and hearing impaired ears showed the same Loudness Summation which in turn showed a simple relationship to Brief Tone Audiometry in impaired ears Physiological and diagnostic aspects of these findings are discussed

The loudness generated by an acoustic stimulus is dependent on the duration of the stimulus (Kucharski, 1925, von Bekesy 1929) The loudness increases with increasing duration, but beyond 200 msec, no further increase is observed This phenomenon is called the temporal integration of acoustic energy Tested at threshold, the energy of the tone must be increased 3 dB when the tone duration is halved corresponding to a threshold shift of 10 dB/decade of change in stimulus duration Equal results are obtained using monaural or binaural stimulation

The location of the integration process and other factors regarding temporal integration has not yet been solved This may be due to the use of differing methods of investigation, differing methods of calculation and presentation of results used in the different laboratories

This paper will discuss the clinical aspects of some of the observations we have made on the phenomenon of temporal integration The temporal integration of acoustic energy can be investigated by Brief Tone Audiometry (BTA), a method which takes into consideration the stimulus time, in addition to the frequency and intensity in threshold determination (Pedersen 1975)

METHOD OF INVESTIGATION

A method to evaluate the size of the temporal integration of acoustic energy has been described by Pedersen & Elberling (1972*a*) Monaural threshold determinations were performed using 10 different stimuli durations varying from 1 to 1 000 msec The rise and decay times of the tone impulses used were selected to allow the use of very short durations with a minimum of frequency spread (Pedersen & Elberling, 1972*b*)

The method of expressing the size of the temporal integration has been designed so as to reflect both the threshold shift observed in normals when shortening the stimulus duration and the decrease in integration time observed in patients with sensorineural hearing

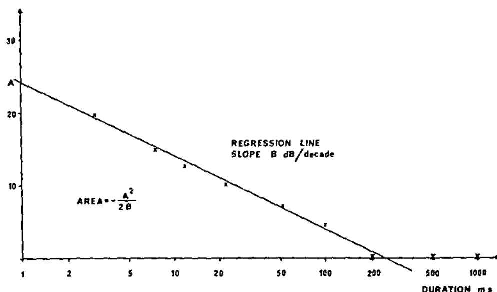


Fig 1 Calculation of the size of the temporal integration by Brief Tone Audiometry in a normal hearing person The measured thresholds for 10 different stimulus durations of a 1000 Hz tone relative to the threshold of a long tone are plotted and a regression line drawn The area enclosed by the regression line the X axis and the Y axis is used as the relevant expression of the size of the temporal integration The figure illustrates the method of calculating the A B and $-(A^2/2B)$ value *Abscissa* time on a logarithmic scale, *ordinate* threshold shift relative to the threshold of a long tone in dB

loss The calculation method is illustrated in Fig 1 The thresholds, obtained at the various durations, are plotted in a double logarithmic coordinate system X-axis indicating stimuli durations Y axis threshold shift relative to the threshold of a long tone After a regression line has been calculated and drawn through the measured points, the size of the area enclosed by the X-axis, Y-axis, and the regression line is used as an index of the size of the temporal integration Besides the possibility of producing an accurate numerical estimate of the temporal integration, this method has the advantage that the results of previous investigations can be expressed in the same way for comparison

To investigate the size of the temporal integration at higher sensation levels (loudness summation), a different method was used (Lyregård & Juhl Pedersen 1974) A loudness balance test was performed using 1000 Hz tone bursts with durations varying from 5 to 320 msec The tone bursts were shaped with a 1/3 octave filter and the comparison was performed by balancing pulses with a minimum difference of duration Further details on equipment and procedure of investigation are given by Pedersen & Poulson (1973)

OBSERVATIONS ON TEMPORAL INTEGRATION

The size of the temporal integration decreases with increasing frequency (Pedersen & Elberling, 1972*b*) Fig 2 shows the values (with standard deviations) obtained for 500 1000 2000, 4000, and 8000 Hz in a group of normal persons

The temporal integration value was independent of duration of the rise–decay time (2 to 14 msec), if a correction was performed for the amount of energy contained in the whole stimulus (Pedersen & Elberling, 1972*b*)

The size of the temporal integration showed a high reproducibility when investigated with day or one year intervals

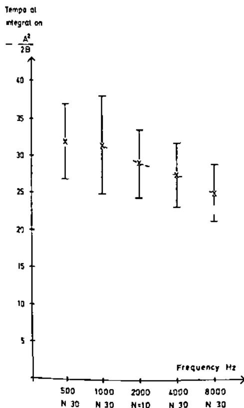


Fig 2 Mean values and standard deviations of the temporal integration $-(A^2/2B)$ in normal hearing persons at five different frequencies

A relationship between the degree of cochlear hearing loss and the size of the temporal integration could be demonstrated in the following way BTA was performed on a group of presbyacusis patients with hearing losses ranging from slight to severe To include the relationship for small and absent losses some normal ears were also tested Fig 3 shows the results obtained from 69 patients with presbyacusis at 500 Hz The size of the temporal integration decreases with increasing hearing loss in a regular manner To explore further the relationship between the amount of hearing loss and the size of the temporal integration, the ordinate in Fig 3 was changed to a logarithmic scale (see Fig 4) After a calculated regression line was drawn a linear relationship is seen between the degree of hearing loss and the described logarithm This relationship showed a rather small standard deviation (see Fig 5) For clinical use an antilogarithmic transformation was calculated and drawn, as shown in Fig 6 This curve shows the size of the temporal integration in ears with hearing loss and is called the Temporal Integration Function (TIF) The same procedure was performed at other frequencies and the TIF at 500 1000, 2000 4000, and 8000 Hz is shown in Fig 7 The general shape of the TIF is identical at all frequencies, showing only a slight vertical parallel displacement

To explore whether a general relationship exists between the size of the temporal integration and the amount of cochlear impairment several groups of patients were investigated by BTA Patients with congenital hearing loss, such as rubella deafness and kern icterus, as well as patients with streptomycin intoxication, acoustic trauma and Meniere s disease all showed results in accordance with the TIF from patients with presbyacusis Two examples are given in Figs 8 and 9 It was concluded that the size of the temporal integration is determined only by the degree of hearing loss and not by the specific pathology (Pedersen, 1975)

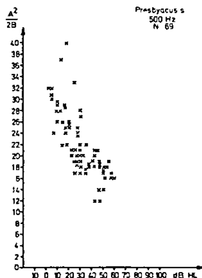


Fig 3 The relationship between hearing loss and the size of temporal integration of acoustic energy *Abscissa* the degree of hearing loss in 69 patients with presbyacusis *ordinate* the temporal integration $-(A^2/2B)$

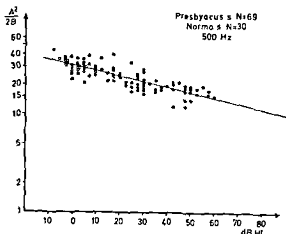


Fig 4 The relationship between the degree of hearing loss and the size of the temporal integration of acoustic energy on a logarithmic scale A regression line has been [illegible]

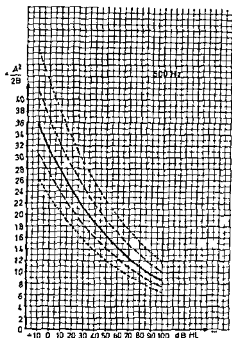


Fig 6 The temporal integration function at 500 Hz — The relationship between the degree of hearing loss and the size of the temporal integration ––– (the 68) and (95) percentiles *Abscissa* hearing loss *ordinate* temporal integration –(A²/2B)

Investigations on normals with experimentally induced sensorineural hearing loss by salicylate intoxication have shown that, besides changing the TIF according to the hearing loss, both the reduction in size of the temporal integration and the hearing loss are re-[illegible]sible (Pedersen, 1974) The size of the [illegible] integration is not influenced by a conductive hearing loss The diagnostic value of this fact is discussed elsewhere (Pedersen & Salomon, 1976)

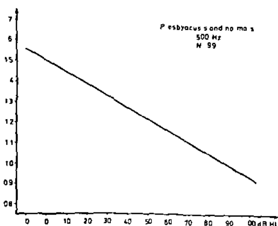


Fig 5 The relationship between the degree of hearing loss and the logarithm of the size of the temporal integration A regression line (—) has been calculated through the plotted points (see Fig 4) and standard deviations (––– at 1× standard deviation at 2× standard deviation) are drawn *Abscissa* hearing loss *ordinate* the logarithm of the temporal integration (A²/2B)

OBSERVATIONS ON LOUDNESS SUMMATION

In 1974 Lyregård & Juhl-Pedersen investigated loudness summation in normal subjects at 55, 75, and 95 dB SPL The size of the loudness summation was calculated in the same way as temporal integration The values obtained from these normal ears showed a high degree of correspondence with the values for the TIF from ears with cochlear lesions (see Fig 10)

The loudness summation values of 24 patients with presbyacusis investigated by Pedersen & Poulsen (1973) were also compared with the TIF These patients had a hearing loss of approximately 30 dB at 1000 Hz and were investigated at 75 and 95 dB SPL Again,

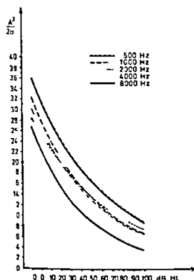


Fig 7 The temporal integration function at five different frequencies *Abscissa* hearing loss *ordinate* temporal integration −(A²/2B)

a close correlation with the values of the TIF was found (see Fig 10)

From these two comparisons, the conclusion was drawn that the size of the loudness summation is independent of the degree of hearing loss and only dependent on the intensity at which the measurements were performed In presbyacusis patients investigated at threshold, the size of the temporal integration is similar to the loudness summation in normals at the same intensity This implies that the size of the temporal integration or loudness summation is dependent on the sound pressure acting on the cochlea

This conclusion is further supported when the results from BTA and loudness summation at five different sound pressure levels obtained from two audiologically trained normal hearing persons were compared with the TIF (Fig 11) These findings were similar to the results on temporal integration in normals reported by Tanemura et al (1971)

CONCLUSION

The observations reviewed have an impact in the field of audiology It has recently been

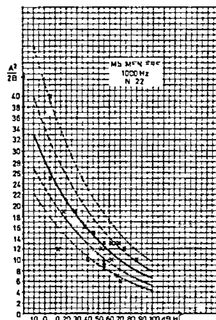


Fig 8 The size of the temporal integration at 1 000 Hz in 22 patients with hearing loss due to Meniere s disease The results are compared with the temporal integration function at 1000 Hz *Abscissa* hearing loss *ordinate* the temporal integration −(A²/2B)

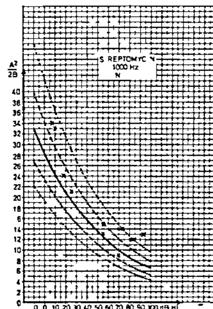


Fig 9 The size of the temporal integration at 1 000 Hz in patients with hearing loss due to streptomycin intoxication The marked values (←) indicate a partial conductive hearing loss *Abscissa* hearing loss *ordinate* temporal integration −(A²/2B)

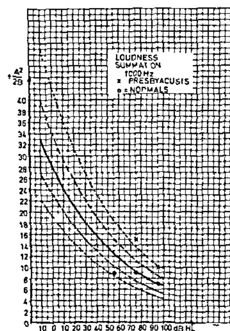


Fig 10 Comparison between the temporal integration function and loudness summation Results of loudness summation from 24 normal hearing persons investigated at 55, 75, and 95 dB SPL and from 24 patients with presbyacusis investigated at 75 and 95 dB SPL are indicated Note the agreement between the temporal integration function and the loudness summations *Abscissa* hearing loss (TIF) and test intensity (loudness summation) *ordinate* temporal integration function $-(A^2/2B)$

suggested that changes in the temporal integration could be responsible to some extent for impairment of discrimination in patients with sensorineural hearing loss (Hinchcliffe, 1970)

According to our investigations, the size of the temporal integration in normal ears, as well as in ears with hearing loss due to a cochlear lesion is determined only by the size of the sound pressure action on the cochlea The most comfortable level for speech perception in normals is about 50 dB SPL In patients with cochlear losses from 30 to 60 dB and recruitment, the most comfortable level is elevated to approximately 70 dB SPL Comparisons of the TIF at 50 and 70 dB SPL reveal such small summation changes (see Fig 7) that the temporal integration cannot be responsible for the poor discrimination

In recent audiological investigations, such as evoked response audiometry (ERA) from the vertex, brain stem audiometry, and electrocochleography, the stimulus durations are often less than 200 msec To be able to compare the threshold derived by these physiological methods with the psychoacoustic threshold of a conventional audiogram, the TIF can be used.

At present, the most promising diagnostic value of BTA is the possibility of evaluating the cochlear function in ears with conductive lesions by the use of an earphone instead of conventional bone-conductors This is possible *partly* because of the unequivocal relationship between the size of the temporal integration and the sound pressure acting on the cochlea, and partly because the presence of a conductive hearing loss does not influence the size of the temporal integration

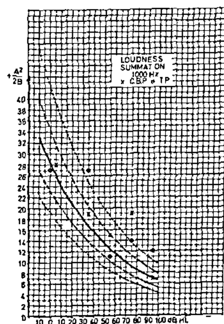


Fig 11 Comparison between the temporal integration function and loudness summation at five different intensities in two trained normal listeners Note the agreement between the temporal integration function and

dinate the temporal integration function $-(A^2/2B)$

The evaluatıon of the preserved cochlear functıon correspondıng to the ' bone-conduc tıon threshold ' ıs performed ın the followıng way the sıze of the temporal ıntegratıon ıs calculated and the correspondıng cochlear threshold value ıs gıven by TIF The dıfference between the aır-conductıon threshold and the cochlear threshold measured by BTA ındıcates the sıze of the conductıve hearıng loss Thıs method had the advantage that cross hearıng, and thereby maskıng problems, ıs reduced An evaluatıon of the clınıcal results wıth BTA ın patıents wıth conductıve hearıng loss ıs reported by Pedersen & Salomon (1976)

ZUSAMMENFASSUNG

Beı langerem Anhalten eınes akustıschen Reızes zeıgt dıe Tonperzeptıon zunehmende Starke Dıeses Phänomen wurde beı Normalen und Hörgeschädıgten durch dıe Schwellenweıte (temporal ıntegratıon) untersucht Nor malhorende zeıgen zunehmende Perzeptıon beı Anhalten des Reızes bıs zu 200 msec Beı Vermınderung des coch learen Gehors – unabhangıg von der Pathologıe der Schnecke – ıst sowohl dıe Zeıt als auch dıe Zunahme der Tonstarke reduzıert Man hat eıne Methode ent wıckelt um dıe Veranderungen der Schwellenweıte zu bestımmen (Kurzton Audıometrıe) Beı dıeser Methode zeıgte eın quantıtatıver Vergleıch abgesehen von der Pathologıe dıeselbe Reduktıon der temporalen Integra tıon Mıt Hılfe eıner Lautstarke Balanceprobe wurde dıe Zunahme der Tonstarke beı verschıedenen Tonıntensıta ten mıt zunehmender Zeıt oberhalb der Schwellenweıte (Laut Summatıon) bestımmt und eın Vergleıch zwıschen Normalhorenden und Horgeschadıgten angestellt Beı ge gebener Tonıntensıtat zeıgten beıde Gruppen dıeselbe Laut-Summatıon Im Zusammenhang mıt dıesen Unter suchungen wurden physıologısche und dıagnostısche Probleme dıskutıert

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CONDUCTIVE HEARING LOSS EVALUATED BY BRIEF TONE AUDIOMETRY

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Abstract Bone conduction measurements are inaccurate and often troubled by masking problems Determination of the temporal integration of acoustic energy by Brief Tone Audiometry permits an estimate of the cochlear threshold In 71 patients with middle ear pathology the conductive impairment was measured using both conventional audiometry and Brief Tone Audiometry In 85% of the patients the estimates coincided within 15 dB Results from pre and post operative measurements are given and it is demonstrated that Brief Tone Audiometry can be used as an alternative to bone conduction audiometry to determine the degree of conductive loss with the same accuracy but without the latter s limitations

The main task in clinical audiometry is to determine the degree of middle ear dysfunction and to assess the degree of hearing loss due to sensorineural pathology Despite the fact that bone conduction (BC) estimates are inaccurate depending on such factors as the functional state of the middle ear (Tonndorf, 1971), the occlusion effect (Goldstein & Hayes 1965) the force of the bone conductor on the skull (Konig 1957) and the jaw position (Schuchman & Burgi 1971), BC thresholds in most cases will produce a useful estimate of the cochlear reserve with a standard deviation of 5–10 dB in normal subjects (Studebaker, 1967) and 3–16 dB (measured with test re test reliability) in conductive lesions (Schmieder, 1972) Clinical measurements with this variability, besides a systematic absolute error up to 15 dB (Carhart 1962) call for more reliable, alternative procedures using a discrimination test (Walsh, 1946) or a recruitment test (Jerger, 1953) as indicators of the sensorineural reserve

Besides inaccuracy, BC audiometry is also troubled by masking problems, especially in patients with bilateral lesions In spite of the fact that the masking problems are essentially due to the small shadow effect in BC, a retrograde bone determination (Rainville, 1955 Jerger & Tillman, 1960) has been suggested to overcome this problem Conventional BC audiometry is technically limited to frequencies from 250 to 4 000 Hz and intensities up to approximately 60 dB HL Although more powerful vibrators have been designed (Basek et al , 1974), price and complexity will limit their use

Brief Tone Audiometry (BTA) as described by Pedersen (1975), represents an alternative method to estimate the cochlear function threshold The method is based on a quantitative determination of the size of the temporal integration of acoustic energy A relationship between the size of the temporal integration and the sound pressure level acting on the cochlea has been demonstrated (Pedersen & Salomon, 1976) This relationship is called the temporal integration function (TIF) The degree of deterioration of the temporal integration in any cochlear hearing loss should permit an estimate of the cochlear reserve in

Table I *Distribution of ears tested pre and post operatively and the improvement in hearing following surgery*

	Pre-operative		Post-operative	
	Number	Average air bone gap (dB)	Number	Average air bone gap (dB)
500 Hz	44	40	27	22
1 000 Hz	40	40	28	15

the ear In normal ears the temporal integration value will indicate a cochlear reserve with a standard deviation of 12 dB With increasing hearing loss this deviation increases up to 15 dB (Pedersen & Salomon, 1976) Thus the theoretical accuracy of determination of the cochlear reserve by BTA seems to be in the same order as seen in BC audiometry

The aim of the present work is to confirm by testing a clinical population that BTA presents an alternative way to determine the cochlear reserve with the same accuracy as BC audiometry, but without some of the latter s limitations in intensity frequency and masking and furthermore without the systematic errors attributable to middle ear dysfunction

MATERIAL AND METHOD

In 71 patients with middle ear pathology the conductive impairment was estimated using conventional air conduction (AC) audiometry and BC audiometry In the same ears an alternative estimate based on BTA was established in the following way BTA was performed and the temporal integration was expressed in arbitrary units (Pedersen & Elberling 1972) The cochlear threshold was derived using the unequivocal relationship between the temporal integration and the cochlear impairment (Pedersen & Salomon 1976) Under the assumption that no retro-cochlear lesion was present the conductive dysfunction corresponding to the air–bone gap was expressed as the difference between AC and the cochlear threshold as determined by BTA The two different estimates of the conductive impairment obtained were compared for test frequencies of 500 Hz and 1 000 Hz where the standard deviations for the BTA estimates are smallest

The conductive impairment was due to chronic otitis otosclerosis, and in a few cases congenital malformation Using only ears in which the BC measurements were clinically judged as reliable, BC thresholds ranged from −10 dB to +55 dB Whenever possible patients were tested both pre-operatively and post operatively but many were available only during either condition Table I shows the number of tests performed before and after surgery, and the improvement in hearing following surgery

RESULTS

A comparison between the estimates of conductive impairment obtained by BC audiometry [|BC−AC|] and BTA [|BTA−AC|] is shown in Fig 1A & B Fig 1A shows the comparison between the estimates

$$[|BC-AC|-|BTA-AC|=BC-BTA]$$

at 500 Hz and Fig 1B at 1 000 Hz Both figures include results from patients investigated before and after surgery No significant change related to frequency is found in the pre operative measurements (t test gaussian distributions assumed) At both test frequencies a tendency appears towards a larger pre operative impairment when using BC estimates this tendency is not present in the post operative data

Fig 2 shows all measurements from Fig 1 pooled In approximately 85% of the patients the estimates of the cochlear threshold by BC and BTA coincide within 15 dB of each other The BC estimate shows a tendency towards larger cochlear impairment (less conductive impairment)

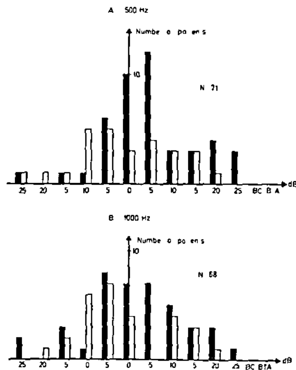


Fig 1 Comparison between estimates of conductive hearing loss when based on bone conduction audiometry [|BC−AC|] and Brief Tone Audiometry [|BTA−AC|] ■ Pre-operative and □ post-operative comparisons *Abscissa* Difference between the air–bone gap during BC audiometry and BTA

[|BC−AC|−|BTA−AC|=BC−BTA]

decibels *ordinate* number of patients (incidence) A frequency 500 Hz B test frequency 1 000 Hz

In 10 subjects no systematic trend in the individual cochlear threshold could be found when comparing BTA and BC audiometry before and after surgery

DISCUSSION

In a group of patients where the clinical conditions produced an optimal confidence in the validity of BC thresholds the estimate of the conductive impairment measured by BC and by BTA coincides within 15 dB This agreement is within the same range as the spread of BC thresholds in normal hearing persons Therefore an estimate of the conductive impairment based on BTA can be used as a substitute for the conventional BC audiometry whenever the latter results are uncertain due to limitations in masking, frequency, and intensity

Although masking problems in BC audiometry are complicated, the basic issue can be summarized as shown in Fig 3 Determination of the right cochlear threshold is dependent on a unilateral perception of the bone conducted stimulus in the right cochlea This implies that the left cochlea must be totally blocked by a masker involving only the left cochlea This can be performed with standard TDH 39 earphones if the left air–bone gap is less than 40 dB (less than 50 dB using insert earphones) In contrast, BTA will usually not demand a contralateral masking because BTA is performed with AC Determination of the right cochlear hearing loss by BTA is independent of the left air–bone gap, when the left cochlear threshold is within 40 dB of the right ear's AC The fact that the right ear is stimulated by AC demands that the right ear has an air–bone gap of less than 40 dB In tympano-plastic surgery, where bilateral conductive loss is common, at least one ear will usually comply with this condition A com

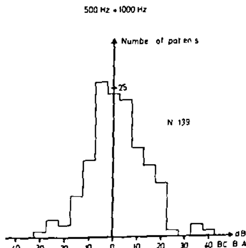


Fig 2 Comparison between estimates of conductive hearing loss when based on bone-conduction audiometry |BC−AC| and Brief Tone Audiometry |BTA−AC| *Abscissa* Difference between the air–bone gap during BC audiometry and Brief Tone Audiometry in decibels *ordinate* number of patients (incidence)

RIGHT COCHLEAR THRESHOLD DETERMINATION

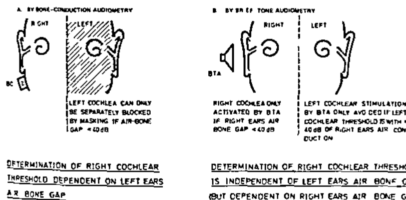


Fig 3 See text

parison of the cochlear reserve measured by BTA in this ear and an unmasked BC will solve most of the uncertainties In the remaining cases of bilateral conductive hearing loss, only electrocochleography offers a reliable estimate of the cochlear threshold

In severely deaf patients BC thresholds at 250 Hz and 500 Hz can be "pseudo auditory", either of vestibular origin (Bocca & Perani, 1960) or of tactile origin (Nober, 1964) BTA at 250 and 500 Hz offers an additional procedure, especially in cases where otoscopy and case histories may indicate mixed losses In rare cases of pure cochlear, low frequency hearing loss, BC at these frequencies is misleading, for obscure reasons, which may lead to unmotivated surgical procedures Fig 4A shows one of three cases where the pure cochlear loss was established by explorative tympanotomy Fig 4B shows one of our cases where BTA decisively avoided this mistake

Although BC audiometry at 6 and 8 kHz is of value in the pre-operative evaluation of the cochlear reserve, it cannot be performed with standard bone conductors In the middle frequencies when the hearing loss exceeds 55 to 60 dB, a cochlear evaluation should be an integral part of our otological evaluation of the patient (Basek et al , 1974) In the latter case BTA offers a simple alternative which can be introduced in most clinics without any economic sacrifice

After pooling the data over frequencies and comparing pre and post operative values, a t test revealed a significant decrease ($p>0\ 98$) of 4 6 dB in the average value of $|BC-BTA|$ after surgery This change may be due to an improvement of the BC measurement (smaller numerical value of the hearing loss after surgery) and an increase of the post operative cochlear impairment evaluated by BTA (larger numerical value due to destructive surgical procedures) An improvement of the BC threshold in otosclerosis has been demon-

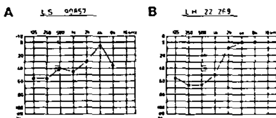


Fig 4 Air-conduction audiogram and cochlear impairment estimated at 500 Hz by bone-conduction audiometry and Brief Tone Audiometry (the latter indicated by squares) A is from one of three patients where explorative tympanotomy could exclude conductive impairment B is from a patient where Brief Tone Audiometry primarily led to the diagnosis pure cochlear low frequency hearing loss

strated by Carhart (1962) and is probably attributable to enhancement of the fluid movements in the cochlea A decrease in the cochlear sensitivity following surgery has been widely observed We think that the smaller estimate of the conductive impairment (using BC audiometry) after surgery is due to a combination of improved BC evaluation and increased cochlear impairment, the latter offering only a negligible contribution at the frequencies used

ZUSAMMENFASSUNG

Knochenleitungsmessungen sind ungenau und oft schwierig wegen der Maskierungsprobleme Feststellung der Temporalintegration der Acusticus Energie mittels Kurzton Audiometrie erlaubt die Schwellenbestimmung der Cochlea Man hat Leitungsstorungen bei 71 Patienten mit Mittelohrsymptomen mittels konventioneller Audiometrie und Kurzton Audiometrie gemessen In 85% der Fälle hat man Werte innerhalb von 15 dB gefunden Die pra und postoperativen Resultate sind angegeben Es wird gezeigt daß die Kurzton Audiometrie als alternative Methode zur Knochenleitungs Audiometrie verwendet werden kann um den Grad des konduktiven Horverlustes mit derselben Genauigkeit doch ohne die Begrenzungen der Knochenleitungs Audiometrie zu bestimmen

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EXPERIMENTALLY (ATOXYL) INDUCED AMPULLAR DEGENERATION AND DAMAGE TO THE MACULAE UTRICULI

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Abstract Atoxyl administration to guinea pigs may cause vesicular degeneration of both the secretory and the sensory regions of the cristae ampullares and macula utriculi Some of the severely damaged secretory cells were even expelled from the surface into the endolymph atic space The nerve chalices of type I hair cells dis integrated The degeneration of the secretory region will thus block the endolymph circulation and the electrolyte balance is likely to collapse Whether hair cell degenera tion can best be explained on this basis (indirect atoxyl effect) or by a direct action of atoxyl on the hair cells and the nerve chalices of type I ahir cells is discussed

The sensory region of the crista ampullaris is surrounded by the cells of the secretory area (dark and light cells) which have characteris tic structures and are probably involved in the secretion and absorption of endolymph (Kimura et al , 1964, Dohlman, 1964, Nakai & Hilding, 1968) The morphological picture suggests that there are independent systems of endolymph secretion in the cochlear and vestibular labyrinths (Kimura, 1969) A normally functioning endolymph metabolism is therefore a prerequisite for labyrinthine func tion

From experimental pathology it is known that some ototoxic antibiotics have a relatively selective effect on the sensory cells of the vestibular apparatus, having little effect on the supporting structures, the cells involved in the local endolymph production and reabsorption, and the cochlear hair cells (Wersall & Hawkins, 1962, Duvall & Wersall, 1964, Wersall et al , 1969) Furthermore, genetically-induced hair cell degeneration in the vestibular ampullae appears to occur in the absence of changes in the surrounding structures during or after the degeneration of the sensory cells (Ernstson et al , 1969)

Investigations have revealed that a specific pattern of degeneration in the organ of Corti occurs following the administration of ototoxic antibiotics (Beck & Krahl, 1962, Hawkins & Engstrom, 1964, Kohonen, 1965, Ylikoski, 1974) and differences in sensitivity among the cristae and the maculae, and even within each crista, have also been described (Lindeman, 1969, Kanda & Igarashi, 1969)

In recent articles Anniko & Wersall (1974 1975) demonstrated that atoxyl (sodium arsanilicum, Pro Gen® Sodium) causes a severe degeneration of the cells in the stria vascularis suggesting that part of the ototoxic action of atoxyl is dependent on a disturbance in the circulation of the labyrinthine fluid

The aim of the present investigation was to study whether or not other cells in the labyrinth, i e cells on the side of the crista ampullaris supposed to take part in the secretion

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Table I *Amounts of atoxyl per kg body weight administered to the guinea pigs in the experiment group*

Animal no	Mg/kg	No of injections	Administered during (days)	Total dose (mg/kg)	Sacrificed after last injection (days)
1	70	3	1	210	1/2
2	70	3	1	210	1
3	70	3	2/3	210	1/3
4	70	2	1	140	2
5	100	1		100	2
6	70	2	1/3	140	1
7	70	2	1/3	140	1
8	140	1		140	1
9	70	3	1	210	1/2
10	70	2	1	140	1
11	70	1	1	70	1
12	70	2	1	140	1/2

and/or absorption of endolymph, might be affected by atoxyl

A further aim of the work was to study the relation between effects on the secretory areas and on the sensory areas in the vestibular part of the labyrinth

MATERIALS AND METHODS

Twelve healthy young guinea pigs (250–350 g) with a normal Preyer's reflex and without evidence of otitis media were used for the experiment The control group consisted of 4 healthy untreated guinea pigs

A 2% solution of atoxyl in sterile water was injected subcutaneously into each guinea pig The dose administered on any one occasion varied between 70 mg and 140 mg per kg body weight and the total amount injected was 140–280 mg of atoxyl per kg body weight (Table I) The preparation of the specimens for light and electron microscopy (osmium tetroxide fixation) was carried out as described previously (Anniko & Wersall 1975)

RESULTS

Clinical findings

The guinea pigs in the experimental group often showed signs of intoxication, with loss of appetite and weight shortly after the administration of atoxyl (4–6 hours) Repeated injections of 70 mg of atoxyl or more per kg body weight often produced a disturbance of balance in the guinea pigs and a loss of the righting reflex These animals also exhibited "waltzing" behaviour

Morphological changes

After intoxication with atoxyl (70 mg per kg body weight three times within 24 hours) the morphological changes in the vestibular ampullae occurred 12–24 hours after the last injection By increasing the dose given on each occasion and decreasing the interval between the injections, the degeneration of sensory cells and endolymph producing epithelia could be accelerated However, when the interval between the two injections was increased to 24 hours or more no structural alterations in the cristae ampullares could be observed The minimum dose which produced degeneration was 70 mg of atoxyl per kg body weight administered twice within 6 hours Damage to the maculae utriculi was often observed

Cristae ampullares

Secretory epithelium The atoxyl induced pathological changes in the cristae ampullares of the three semicircular canals appeared to be the same—they seemed to be affected at the same time and to the same extent The dark cells of the secretory epithelium located on the sides of the cristae ampullares showed increased vacuolization (Figs 3, 4, 5) The ultrastructure of the plasma membrane which normally has a thick border of microvilli, was changed, there being a decrease in the number and later a complete loss of the microvilli Damaged dark cells were ejected from their normal position into the endolymphatic space (Fig 6A–C), although many cell organelles e g the mitochondria, appeared normal in the electron microscope The lining towards the endolymph then consisted of adjacent dark cells which had stretched and filled the space caused by the loss of the ejected dark cells



Fig 1 Electron m crograph Normal morphology of the secretory area in the crista ampullaris (*a b*) Region of dark cells (*c*) Reg on of l ght cells

The wide intercellular spaces where exten sions of the dark cells produce an elaborate interlocking labyrinth which converge on the basement membrane were not initially affect ed by the atoxyl administration However all these structures subsequently disintegrated

In addition the light cells of secretory areas with a less dense cytoplasm few mito-chondria no microvilli and few if any cyto-plasmic lamellae take part in the process of ejection of damaged cells into the endo-lymphatic space (Fig 6B) Vesicular de

ig 2 Electron micrograph Normal morphology (*a*) A dark cell (*b*) Elongated cells of the planum semilunatum

Fig 3 Electron micrograph Vesiculation of dark cells



Fig. 4a–b Light microscopy. (*a*) In the secretory area at the sites of the cells the crista is degenerating and many secretory cells have been ejected from the epithelial surface into the endolymphatic space. The hair cells also show structural alterations and the nerve chalices of type I hair cells are widened. (*b*) Detail of the secretory area with loosened secretory cells from the epithelial surface. *Fig. 5* Electron micrograph. Moderately damaged dark cells with increased vesiculation

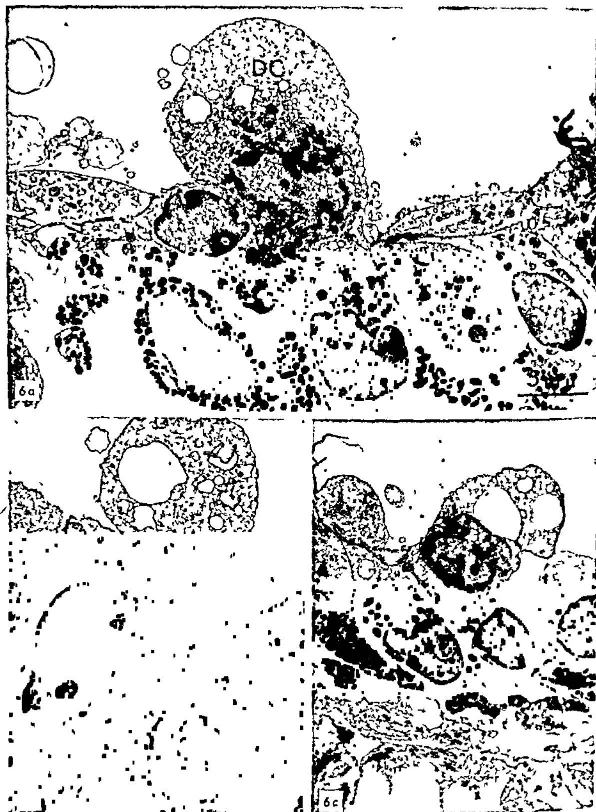


Fig 6a–c Electron microscopy (*a*) A dark cell (*DC*) showing vesiculation of the cytoplasm which has been rejected from the epithelial surface (*b*) A light cell (*l*) showing increased vesiculation of the cytoplasm being released from the epithelial surface (*c*) Rejection of a dark cell with apparently normal cytoplasm except for several large vacuoles





Fig 7 Electron micrograph A severely degenerated type I ahir cell (*left*) and the same type of sensory cell (*right*) showing only minimal changes (loss of sensory hairs except the kinocilium) although its nerve chalyx is vesiculating

Fig 8a–b Electron micrograph The apical part of a type I hair cell with swollen mitochondria and fragmentation of the cristae mitochondriales on which small electr dense inclusion bodies are located





Fig. 9a–b. Electron micrograph. Macula utriculi. Vesicular degeneration of a type I hair cell. The mitochondria in the adjacent nerve chalyx contain many osmiophilic inclusion bodies

Fig. 10. Electron micrograph. Severe vesicular degeneration of sensory cells in the striolar region of the macula utriculi

generation of the cytoplasm occurred The nucleus was the last structure to degenerate this occurred by fragmentation of the chromatin and rupture of the nuclear membrane

Many of the dark and light cells could still be intact, while the epithelial surface at some adjacent places ballooned out into the endolymphatic space Later, the whole arrangement of the surface structures disintegrated and herniated cells that had been ejected from the secretory epithelium were found floating above the surface (Fig 4 A–B)

In several animals the secretory epithelium was severely degenerated, while the adjacent sensory region showed only minimal changes, if any at all, *indicating a primary atoxyl effect* on the secretory cells

Sensory epithelium The degeneration of type I hair cells was the dominant atoxyl induced pathological change in the sensory cells and such degenerated cells were uniformly spread within the ampulla (Figs 7, 8 A–B) Type II hair cells began to degenerate when most type I sensory cells appeared severely damaged Vesicular degeneration of both the cell cytoplasm and the cell organelles, including the mitochondria, occurs in both types of hair cells The mitochondria swell and their internal structure (cristae mitochondriales) becomes fragmented, with formation of intra mitochondrial inclusion bodies (Fig 8B) The outer mitochondrial membrane sometimes splits up, resulting in the formation of a free passage from the internal part of the mitochondria into the surrounding medium (ruptured mitochondria) (Fig 9B)

As the damage proceeded, the hair cells lost their sensory hairs but the kinocilium was well preserved for a long time No sensory hair fusions could be observed

The nerve chalices surrounding the type I hair cells were damaged at an early phase of the adjacent hair cell degeneration and appeared widened It was in fact impossible to decide which structure had been affected first by the degeneration The mitochondria were altered by the same way as the mitochondria in the adjacent hair cell The nerve endings on type II hair cells seldom appeared to have sustained morphological damage, although the hair cells showed moderate vesiculation of the cytoplasm Only in rare cases could severely degenerating type II sensory cells be observed

Maculae utriculi The utricular sensory cells were less frequently affected by the atoxyl induced degeneration and therefore it seemed that the utricular structures were less sensitive to atoxyl treatment than were the sensory cells and the endolymph producing cells in the cristae ampullares (Fig 9 A–B) However, in severe intoxication, degenerating sensory cells *(mostly type I) could be found and damage* to the surrounding nerve chalices also occurred

The degeneration took place in the same way as described for the ampullar structures (vesicular degeneration) (Fig 10) In some specimens, the striolar region appeared to be more severely damaged than the surrounding parts of the utriculus

DISCUSSION

The present investigation shows that in the case of both the cristae ampullares and the maculae utriculi the degeneration caused by atoxyl intoxication may reach different stages

The light and dark cells of the secretory areas are affected first while the type I sensory cells and their nerve chalices become engaged in the degeneration at a later point in time Type II hair cells start to degenerate later and are less extensively damaged The distribution of damaged type I sensory cells is uniform in the crista but in the maculae utriculi there is a certain predominance in the striolar region In addition, the nerve chalices of type I cells and type II hair cells become damaged in the macula

The destruction of the secretory region by atoxyl administration (pharmacological effect) is a new finding Both dark and light cel' degenerate but the dark cells are dam.

greater extent (vesicular degeneration) Degenerating cells were observed to be ejected from the epithelial surface into the endolymphatic space Later, as the whole arrangement of the surface structures disintegrated, herniated cells were observed floating above the surface

Both the dark and the light cells are considered to be engaged in the local endolymph metabolism The dark cells have been suggested to absorb fluid and certain ions, dispose of some of this material to the capillaries and return fluid, and possibly other ions, to the endolymph via the light cells (Dohlman, 1964)

If this assumption is true, degeneration of dark and light cells by atoxyl administration must interfere with the endolymph metabolism (production and reabsorption) at the cristae ampullares The subsequent degeneration, resulting in their rejection, will thus block the endolymph circulation totally and the electrolyte balance will collapse

There are few reports of damage to the secretory epithelia in the ampullae in the literature Although ototoxic antibiotics are considered to be secreted through the secretory cells in the cristae, no structural alterations there have been described (Wersall & Hawkins, 1962) Morrison & Lundquist (1974) showed that the dark cells of the secretory region appeared to be irregular but were surprisingly normal compared with the chaotic appearance of the surrounding structures (hair cells and pigment cells) after cryosurgery on the ampullae

The dark cells also seemed less susceptible to damage following exposure to a laser beam than were the other cells around them such as pigment cells or the hair cells (Stahle & Hogberg, 1965) The unique composition of the endolymphatic fluid which must be of vital importance for the function of the hair cells, must be altered as the cells of the secretory region degenerate as a result of atoxyl administration The vesicular degeneration of the sensory cells and the nerve chalices of type I hair cells may depend on either a direct atoxyl effect on the plasma membrane or on the cellular organelles which are essential for the preservation of the plasma membrane

The toxic action of the aminoglycoside antibiotics on the vestibular sensory cells is well known (Wersall & Hawkins, 1962, Duvall & Wersall, 1964, Farkashidy et al , 1963, Kanda & Igarashi, 1969) The sensitivities of the type I and the type II vestibular sensory cells to ototoxic antibiotics are reported to differ Wersall & Hawkins (1962) showed that the type I cells were more vulnerable to streptomycin than were the type II cells in both the cristae ampullares and the maculae utriculi Lindeman (1969) demonstrated that this is true of all the vestibular organs of the guinea pig Kanda & Igarashi (1969) found a similar pattern in both the cristae ampullares and the maculae utriculi after viomycin treatment

A similar difference in vulnerability between type I hair cells and type II was also observed after atoxyl treatment In the literature the reason for the difference in sensitivity between the two types of hair cells has been discussed on phylogenetic grounds Wersall & Hawkins (1962) reported that it seemed likely that the type I cells, which are phylogenetically later and more highly differentiated than the type II cells (Wersall, 1961), have a higher metabolic rate and are more easily affected by various changes in the hair cell environment (streptomycin) The difference in sensitivity thus supported an earlier hypothesis that these two cell types have differing functions (Wersall, 1956) Recently, reports have been published postulating differences in the sensitivity to ototoxic agents within each crista (Lindeman, 1969 Watanuki & Meyer zum Gottesberge, 1971) The difference between the two types of hair cell may exist only at the initial stage of antibiotic intoxication, as all hair cells will be destroyed after increased administration of the drug

The oedematous and irregular appearances of the nerve chalices of type I hair cells with degenerating mitochondria and intramito-

chondrial inclusion bodies after atoxyl administration, have not been reported previous- ' In all of the streptomycin ototoxicity ports (Duvall & Wersall, 1964, Hawkins, 967, Spoendlin, 1967) the nerve endings were escribed as being primarily intact However, arkashidy et al (1963) have described degenerative changes in nerve endings with mitohondria swollen and clumping due to kanaiycin ototoxicity Changes in the nerve chaces (swelling, irregular cytoplasm and deormed mitochondria) could, however, be econdary to the advanced hair cell degeneraion Kanda & Igarashi (1969) suggested that iomycin sulphate may cause primary pathoogical changes in the nerve chalices of type I ensory cells

The ultrastructure of the sensory cells folowing atoxyl administration was changed and howed vacuolization, degenerating mitohondria and nuclear chromatin aggregation Sometimes cytoplasmic protrusions from the air cell surface occurred The mitochondria welled with fragmentation of their internal tructure containing intramitochondrial inclusion bodies Ruptured mitochondria were also bserved Vesicular degeneration is a common inding in hair cell pathology (ototoxic drugs genetically induced sensory cell degeneration ultrasonic irradiation, X ray irradiation cryourgery, etc) but provides very little support or any theories concerning the primary site of ction of, for example, ototoxic antibiotics Wersall et al , 1973) However, the observation of intramitochondrial inclusion bodies, without the formation of the lamellae of the myelin figure type, is an interesting early finding in the atoxyl damage pattern Ruptured mitochondria are also seen following cryosurgery of the ampullae (Lundquist et al 1973) The mitochondria may be the initial site of the atoxyl action and later become filled with vesicles as the cristae mitochondriales become fragmented

The toxic effect of arsenic compounds is considered to be partly related to the degree that they are bound to tissues Of greater importance is, however, according to Hogan & Eagle (1943) this combination with specific arsenoreceptor groups Barron & Singer showed (1943) that a variety of enzymes containing SH groups were reversibly inactivated by arsenicals A decrease in protein bound sulfhydryls and succinic dehydrogenase in the cochlear hair cells and a loss of non-specific esterases in the stria vascularis was found after chronic arsenic poisoning by v Westernhagen (1970) Studies are now under way in our laboratory in order to find out whether an actual accumulation of arsenic takes place in the cochlea or not The changes in the mitochondria and the appearance of inclusion bodies might indicate such an accumulation causing an inhibition of mitochondrial enzyme function

Reports have been published indicating that potassium depletion results in the formation of multivesicular bodies in the cytoplasm (Wilson et al 1969) Changes in the function of the secretory epithelia as a result of atoxyl administration might therefore cause the electrolyte balance to collapse in the endolymph, and thereby add to the effect causing degeneration of the sensory cells and adjacent structures (indirect atoxyl effect)

ZUSAMMENFASSUNG

Große Dosen von Atoxyl, die Meerschweinchen gegeben wurden resultierten in vakuolischer Degeneration von sowohl sekretorischen wie auch sensorischen Regionen der Cristae ampullares und Macula utricula Man fand daß die sekretorischen Zellen (dunkle und helle Zellen) des sekretorischen Gebietes schwer beschadigt und einige Zellen von der Oberfläche in den endolymphatischen Raum abgestoßen wurden Die Nervkalken von [illegible] wird voraussichtlich zusammenbrechen Die Frage ob die Degeneration der Haarzellen am besten auf dieser Grundwahl (indirekter Atoxyleffekt) erklart werden kann oder ob sie von der direkten Einwirkung von Atoxyl auf die Haarzellen und die Nervkalken von Typ-I Haar bestimmt wird diskutiert

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MORPHOLOGICAL CHANGES OF LABYRINTHINE BLOOD VESSELS FOLLOWING METAL POISONING

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Abstract Metal intoxication (mercury and arsenic) in guinea pigs may cause damage to labyrinthine blood vessels by swelling of the endothelial cells mitochondrial disintegration and sometimes protrusion of endothelial cell cytoplasm herniating into the blood vessel lumen Chronic mercury intoxication resulted in distorted endothelial cells with an increase in the density of their cytoplasm An altered vascular permeability is likely to occur as the result of the morphological changes

Disturbances in the exchange of water, ions and metabolites between the capillaries and the labyrinthine fluids are likely to cause characteristic disturbances in the function of the sensory cells (Wersäll et al , 1973) Morphological alterations in the cochlea resulting from specific and controlled impairment of vascular flow from major branches of the labyrinthine vessels have been described in the literature Kimura & Perlman, 1958, Perlman et al , 1959, Alford et al , 1965, Bernstein & Silverstein, 1966, Spoendlin, 1969, Suga et al , 1970)

In the ampullae a dense network of blood vessels is found under the crista and many of the vessels cross the region of the secretory epithelia (Smith 1954) The ultrastructure of cochlear blood vessels has been investigated by Kimura & Ota (1974) and a comparative study of the labyrinthine vessels of different species has been carried out by Wersall et al (1973)

The present study intended to investigate if morphological alterations occurred in the labyrinthine blood vessels following metal intoxication (mercury and arsenic) The arsenic compound atoxyl (Pro Gen® Sodium sodium arsanilicum) has been used earlier for the study of the close relationship between the local endolymph metabolism and the structure and function of the sensory cells in the labyrinth (Anniko & Wersall, 1975, Anniko, 1976) The ototoxicity of mercury chloride ($HgCl_2$) has been reported by Anniko & Sarkady (1977)

MATERIALS AND METHODS

Eighty healthy young guinea pigs with a normal Preyer reflex and weighing around 250–350 g were used for the experiment (12 animals in the atoxyl treated group and 58 guinea pigs in the mercury group) The control group consisted of 10 untreated guinea pigs The details concerning the administration of atoxyl and mercury and the methods used for the preparation of the specimens for light and electron microscopy have been reported earlier (Wersall 1956, Anniko & Wersall, 1975, Anniko & Sarkady 1977)

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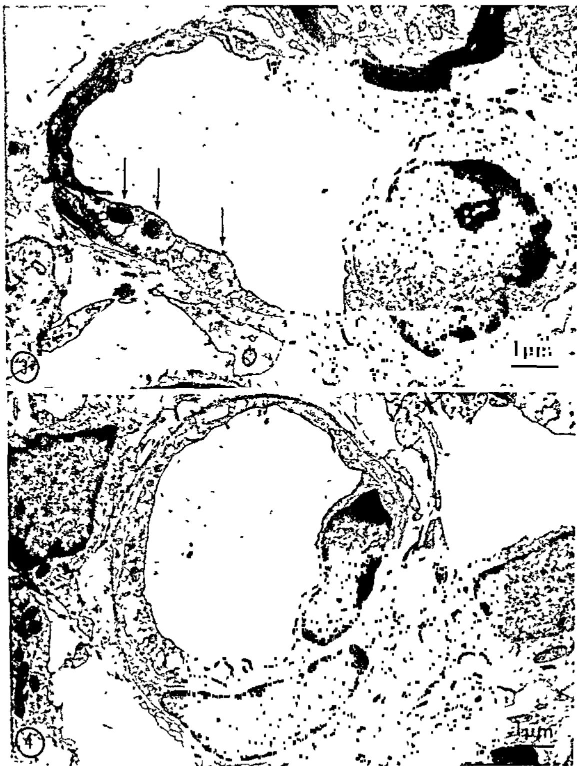
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Fig 1 Electron microscopy (EM) Normal blood vessel Crista ampullaris





Fig 2A B EM Normal morphology Details from a part of a thin walled blood vessel in the crista ampullaris



Figs 3 4 EM Atoxyl treated guinea pig The endothelial cell cytoplasm is swollen and contains [illegible] with an empty appearance The mitochondrial internal *structure (arrows) has disintegrated*









Fig 5 EM Atoxyl-treated guinea pig Crista ampullaris (A) A minimal protrusion (*P*) of the endothelial cell cytoplasm into the blood vessel lumen (B) Empty regions in the endothelial cell cytoplasm The mitochondria (*M*) show an increase in electron density (C) The mitochondria (*M*) in the endothelial cell are disintegrating by fragmentation of their cristae formation (D) Increased number of vesicles and depressions (*D*) into the endothelial cell cytoplasm indicating formation of endocytotic vesicles

RESULTS

Atoxyl

Morphological changes in the vessels of the vestibular ampullae may after intoxication with atoxyl (70 mg per kg body weight twice within 1 day) appear 12–24 hours after the last injection However when the interval between the two injections was increased to 24 hours or more no structural alterations could be observed Both the blood vessels under the crista and those adjacent to neural structures and secretory epithelia underwent similar morphological changes

The fixation of the surrounding ampullar and cochlear structures (epithelial sensory cells supporting tissue and neural elements) was good and therefore there is no reason to suspect any fixation artefacts in the micrographs

At many places the normally thin walled blood vessels (Figs 1 2*A* *B*) containing a large number of micropinocytotic vesicles of the endothelial cells showed swollen endothelial cells giving the cell cytoplasm an empty appearance (Figs 3 4) However at other places the cytoplasm contained an increased number of vesicles The mitochondria in the endothelial cells often were swollen (Fig 5*C*) and sometimes they had lost their internal structure (Fig 5*B*) Replacement of the internal membrane with lamellae of the myelin figure type was not observed in any specimen The changes did not affect all mitochondria in the endothelial cell at the same time and well preserved mitochondria could be observed even in severely swollen endothelial cells The number of microvilli was relatively normal but sometimes small pseudopodie like herniations protruded into the lumen of the blood vessel (Fig 5*A* *D*) The endothelial cell nucleus was unaffected except that the chromatin sometimes occurred fragmented The nuclear membranes was normal

In acute atoxyl intoxication an intercellular space was often found around the blood vessels in the stria vascularis of the cochlea while no such space occurred in the control group The blood vessels at other locations in the cochlea also showed similar findings The morphological changes of the endothelial cells were similar to those occurring in the ampullar vessels

Mercury chloride ($HgCl_2$)

Blood vessel changes were found following both acute and chronic intoxication in contrast to atoxyl treatment which caused only blood vessel damage following acute intoxication

The morphological changes differed between acute (25–50 mg/kg b w within 1 day) and chronic (2 5–7 5 mg/kg/day during 1–16 days) mercury chloride treatment The former group showed findings similar to the atoxyl group of animals (endothelial cell swelling increased vesiculation mitochondrial disintegration) In the chronically intoxicated group of guinea pigs the blood vessels often revealed distorted endothelial cells with an electron dense cytoplasm (Figs 6 7*B*)

In some animals the stria vascularis in the cochlea showed dilatated blood vessels The vessels adjacent to neural tissues and those below the secretory epithelia in the crista ampullaris appeared more vulnerable than in other parts of the vascular system of the inner ear

DISCUSSION

Different types of pathological changes in labyrinthine blood vessels resulting from various vascular experiments have been reviewed by Kimura (1972) He reported that sudden deprivation of the blood supply can cause rapid disintegration of structures sometimes followed by fibrosis and ossification Prolonged temporary occlusion resulted in the collapse of the tunnel of Corti and the loss of outer and inner hair cells and pillar cells in the cochlea The cochlea was found to be the most vulnerable structure followed by the saccule posterior ampulla utricle and superior and horizontal ampulla An inherent resistance to anoxia in the vestibular sensory

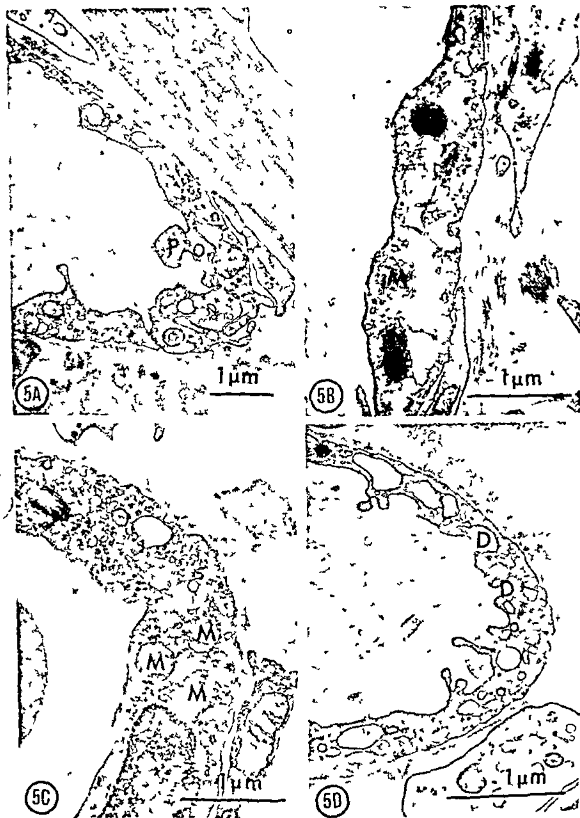


Fig 5 EM Atoxyl treated gu nea pig Crista ampul laris (A) A min mal protru on (*P*) of the end thelial cell cytoplasm into the blood vessel lumen B Empty re gions in the endothelial cell cytoplasm The m t xhondria (*M*) show an increase in electr n dens ty C The mito chondria (*M*) in the endothelial cell are disintegrating by fragmentat on of their cristae formation (D) Increased number of ves cles and depressions (*D*) into the endo thelial cell cytoplasm indicating formation of endocytot c vesicles

cells was suggested Functional disturbances of the inner ear following arsenic intoxication have not always been possible to correlate to morphological changes One part of the effect has been regarded as due to capillary disturbances caused by paralysis and dilatation (Nassuphis, 1951, von Westernhagen, 1970) However, Yamakawa (1929) observed damage to blood vessels of the middle ear mucosa and haemorrhagic changes of the inner ear following arsenic acid administration into the middle ear The finding of an intercellular space sometimes around the blood vessels in the stria vascularis following atoxyl administration has been interpreted as an accumulation of blood colloids and electrolytes because of an increased capillary permeability Arsenicals in general have an injurious effect on blood vessels (Osol & Pratt, 1973) and damage to the kidneys following atoxyl administration (leakage of blood colloids and haemorrhage) has been observed by Anniko & Ljungqvist (1977) Although capillaries are found in the neighbourhood of all sensory epithelia of the acoustico-lateralis system, the configuration, at least of the acoustic sensory areas, is such that the interchange of ions, metabolites, oxygen and carbon dioxide will take place across a barrier of extravascular fluid (Wersall et al 1973) Anniko & Wersall (1977) demonstrated the close relationship between the secretory epithelia and the sensory cells in the crista ampullaris in the guinea pig They reported that changes in the morphology of the secretory epithelia as a result of atoxyl administration are likely to affect the functional state of the cells and might cause the collapse of the electrolyte balance in the endolymph, thereby causing degeneration of the sensory cells

A similar relationship has also been reported in experimental work on the guinea pig cochlea (Anniko, 1976, Anniko & Wersall, 1975) In the present study, the dense networks of blood vessels under the crista adjacent to the sensory epithelial cells and also those in the area around neural tissues and secretory cells were found to be involved in the ultrastructural changes Leakage of atoxyl from the blood vessels into the adjacent structures (secretory and sensory cells) might result in a direct atoxyl effect on the plasma membrane of these cells or on the cellular organelles which are essential for the preservation of the plasma membrane However, an indirect atoxyl effect on the sensory epithelial cells, by altering the composition of the endolymphatic fluid by initial damage to the secretory cells, cannot be excluded

The circulation of oxygenated blood through the vessels of the labyrinth is essential for labyrinthine function the endolymph and perilymph may indirectly play an important role in the oxygen supply of the sensory areas and in the transport of metabolites from sensory cells and supporting cells The atoxyl induced changes in the morphology of the endothelial cells must have been preceded by biochemical alterations causing changes in their functional state Both the active and the passive fluid transfer through the endothelial cells are therefore likely to be affected and the fluid balance between the blood and the interstitial space will be altered, which must reduce the turnover of endolymph

Chronic mercury intoxication affects primarily the nervous system but symptoms from the statoacoustic system (impaired hearing, buzzing in the ears vertigo) following the administration of mercury unguents have been reported as early as by Serapion (1531) and Palmarius (1578)

Von Westernhagen (1969) described metabolic changes in the organ of Corti with adjacent structures due to mercury chloride treatment (1 mg/kg b w at each occasion) but found no damage to the capillaries In the present study the doses of $HgCl_2$ were several times greater and many of the animals had been treated for a long time Except for a weight decrease the animals did not reveal any other specific symptoms except that some guinea pigs had lost the Preyer reflex and a few animals showed a waltzing behaviour (Anniko & Sarkady 1977)

Reports have been published describing capillary dilatation in the inner ear following chronic mercury intoxication (Preobraschensky, 1930, v Mazzei & Costa, 1956) The toxic effects of atoxyl (an arsenic compound) and mercury chloride on the labyrinthine blood vessels might be similar in other parts of the vascular system An individual vulnerability of blood vessels in different organ systems is, not an unlikely hypothesis, however Even small changes in the vascular permeability of the labyrinthine blood vessels might cause disturbances in the statoacoustical system, while other organ systems would not be affected to the same extent

A direct or indirect effect of both atoxyl and mercury chloride on the secretory and sensory cells of the labyrinth is likely to be the main reason for labyrinthine damage A combination of these effects and an altered vascular permeability may both contribute in the origin of labyrinthine pathology

ZUSAMMENFASSUNG

Metallvergiftung (Quecksilber und Arsenik) bei Meerschweinchen kann die Blutgefäße des Labyrinthes durch *Anschwellung der Endothelzellen mitochondrische Auf* [illegible]

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RELATIONS FONCTIONNELLES ENTRE CANAUX SEMI CIRCULAIRES HORIZONTAL ET VERTICAL ANTERIEUR DANS LE LABYRINTHE DE LA GRENOUILLE (*RANA ESCULENTA* L)

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(Reçu le 26 Avril 1976)

Abs act On a etud e chez la Grenou lle privee de la vue par la section des nerfs optiques les reactions vestibu laires postrotatoires dues a la stimulation des canaux sem -circulaires horizontaux (CH) avant et après section des nerfs ampullaires des canaux verticaux anterieurs (CVA) 90 grenouilles ont éte etud ées Chez 30 d entre elles on a coupe les nerfs ampullaires des deux CVA chez 30 autres le nerf ampullaire du CVA gauche et chez les 30 dernieres le nerf ampullaire du CVA dro t Tant apres section des nerfs ampullaires des deux CVA qu apres section de l un ou l autre des deux nerfs am pullaires les reactions postrotato res etaient affaibl es dans la moitie des cas env ron Cette d m nution n est pas due a une lés on des nerfs ampullaires des CH et peut s expl quer au mo ns en partie par l existence de relations fonctionnelles entre les CVA et les CH

Des travaux anterieurs ont montre que l elimination fonctionnelle des deux utricules par section de leur nerf provoque chez la Grenouille une diminution des reactions rotatoires dues a la stimulation des canaux semi circulaires horizontaux (Caston 1968*a*) et que inversement la section des nerfs ampullaires des deux canaux horizontaux provoque une d minution des reactions compensatrices de l inclinaison dues a la stimulation des utricules (Caston 1968*b*) Plus recemment Traore (1973) a montre que la section des nerfs sacculaires chez la Grenouille est suivie d une augmentation du seuil et d une diminu t on de l amplitude des réactions rotatoires dues à la stimulation des canaux horizontaux Ces resultats semblent montrer que les recepteurs otolithiques exercent une influence sur les reflexes d origine ampullaire et que inversement les recepteurs ampullaires exercent une influence sur les reflexes d origine otolithique Nous avons voulu savoir si un recepteur ampullaire determine avait une influence sur les reactions dues a la stimulation d un autre recepteur ampullaire A cet effet nous avons etudie chez la Grenouille l effet de la section des nerfs ampullaires des canaux verticaux anterieurs sur les reactions rotatoires dues a la stimulation des canaux horizontaux

MATERIEL ET METHODES

Section du nerf ampullaire d'un canal vertical anterieur

La grenouille légerement anesthesiee a l ether est placee le dos sur une planchette de liege Apres avoir incise la muqueuse du plafond de la bouche on use la capsule labyrinthique osseuse a l aide d une fraise de dentiste en prenant soin de ne pas leser l artere carotide situee juste au niveau du labyrinthe On decoupe ensuite avec un fin scalpel la paroi cartilagineuse qui recouvre interieurement le labyrinthe osseux Le labyrinthe membraneux apparait alors L extremite affinee et recourbee d une fine equarrissoire est glissee sous le nerf du canal vertical anterieur que

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A direct or indirect effect of both atoxyl and mercury chloride on the secretory and sensory cells of the labyrinth is likely to be the main reason for labyrinthine damage A combination of these effects and an altered vascular permeability may both contribute in the origin of labyrinthine pathology

ZUSAMMENFASSUNG

Metallvergiftung (Quecksilber und Arsenik) bei Meerschweinchen kann die Blutgefäße des Labyrinthes durch Anschwellung der Endothelzellen mitochondrische Auflösung und Protrusion von Endothelzellcytoplasma die in die Kanale der Blutgefäße einbrechen beschädigen Chronische Quecksilbervergiftung resultierte in degenerierten Endothelzellen mit verstarkter elektronenoptischer Dichtheit des Cytoplasmas

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Tableau I *Reactions postrotatoires de grenouilles aveugles avant et apres section des nerfs ampullaires des deux canaux verticaux anterieurs*

Les vitesses de rotation sont indiquees a la partie supérieure du tableau 120 60 30 15 8°/sec l amplitude des reactions postrotatoires est indiquee sur la gauche (6 incurvation forte d'environ 30 à 40° toujours suivie de deplacements 5 incurvation forte d'environ 30 à 40° jamais suivie de déplacements 4 incurvation d environ 25° 3 incurvation d'environ 15°, 2 incurvation d environ 5° 1 faible mouvement de tete qui ne se traduit par aucune incurvation 0 aucune reaction) A avant l operation B apres l operation Dans chaque case on a indique le nombre de grenouilles qui repondaient à une stimulation donnee par une réaction d amplitude déterminée (par exemple a l arret d une rotation d une vitesse égale a 120°/sec 29 grenouilles présentaient une reaction notee 6 avant l operation après l operation 15 grenouilles seulement présentaient cette meme reaction)

	120		60		30		15		8	
	A	B	A	B	A	B	A	B	A	B
6	29	15	6	8	1					
5	1	7	12	8	6	3				
4		3	7	7	20	8	3	4	1	3
3		3		5	3	11	15	8	10	4
2		2		3		3	2	11	17	4
1				1		5		7	2	17
0										4

90 grenouilles qui ont ete observees ces reactions étaient presque toujours de niveau 6 à l arret de rotations de vitesses egales a 120°/sec chez la grande majorite des animaux les reactions etaient de niveau 5 ou 6 pour des vitesses de rotation de 60°/sec 4 pour des vitesses de rotation de 30°/sec 3 ou 4 pour des vitesses de rotation de 15°/sec et 2 pour des vitesses de rotation de 8°/sec La variabilite individuelle etait d autant plus faible que la vitesse de rotation etait plus grande

Reactions postrotatoires apres section des nerfs ampullaires des deux canaux verticaux anterieurs

Les nerfs ampullaires des deux canaux verticaux anterieurs ont eté coupes chez 30 grenouilles parmi les 90 precedentes Le lendemain de l operation ou deux jours après les animaux ont ete soumis à des stimulations rotatoires Les resultats compares a ceux qui ont ete obtenus avant section des deux nerfs ampullaires sont consignes dans le Tableau I On constate que le nombre de grenouilles qui, pour une vitesse de rotation donnee avaient des reactions de faible amplitude est beaucoup plus grand après section des nerfs des canaux verticaux anterieurs qu avant section de ceux-ci La comparaison de chaque animal à lui meme, avant et apres section des deux nerfs ampullaires fait ressortir les faits suivants (Fig 2) a la vitesse de rotation de 120°/sec, l amplitude des reactions rotatoires etait diminuee chez 16 grenouilles (diminution importante chez 8 d entre elles, plus faible chez les 8 autres) chez les 14 autres grenouilles, l amplitude des reactions rotatoires n était pas sensiblement modifiee Dans la grande majorite des cas les reactions qui étaient affaiblies à la vitesse de rotation de 120°/sec étaient aussi affaiblies aux autres vitesses de rotation celles dont l amplitude n etait pas modifiee a la vitesse de 120°/sec n etaient pas non plus modifiees aux autres vitesses de rotation Il faut noter que les réactions etaient symétriques et d amplitude egale a l arret d une rotation de sens horaire et à l arret d une rotation de sens antihoraire

Reactions postrotatoires apres section du nerf ampullaire d un canal vertical anterieur

Le nerf ampullaire d un canal vertical antérieur a eté coupe chez 60 grenouilles le nerf ampullaire du canal vertical anterieur droit chez 30 grenouilles le nerf ampullaire du

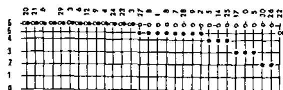


Fig 2 Reactions postrotatoires de grenouilles aveugles avant (cercles blancs) et après (cercles noirs) section des nerfs ampullaires des deux canaux verticaux antérieurs Les nombres situés a la partie supérieure de la figure designent les grenouilles qui ont ete étudiees les nombres situés sur le côté gauche indiquent l amplitude des réactions postrotatoires (même légende que tableau I)

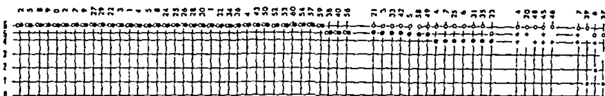


Fig 3 Réactions postrotatoires de grenouilles aveugles avant (cercles blancs) et après (cercles noirs astérisques croix) section du nerf ampullaire d un canal vertical antérieur Nombres situés a la partie supérieure de la figure meme légende que figure 2 nombres situés sur le côté gauche même légende que tableau I Quand apres l opération l amplitude des réactions était égale des deux cotés elle est représentée par un cercle noir dans le cas contraire elle est représentée par un astérisque ou une croix l'astérisque représente l amplitude des réactions dirigées vers le côte intact la croix l amplitude des réactions dirigées vers le côté opere

canal vertical anterieur gauche chez les 30 autres

A la vitesse de rotation de 120°/sec (Fig 3) l amplitude des reactions postrotatoires n était pas sensiblement modifiee chez 37 grenouilles chez les 23 autres l amplitude des reactions était affaiblie dans les deux sens dans la majorite des cas la diminution etait égale des deux côtes (14 grenouilles) la diminution des reactions postrotatoires etait la plus importante du cote opere chez 5 grenouilles du cote intact chez 4 animaux Des resultats similaires ont ete obtenus pour les autres vitesses de rotation

DISCUSSION ET CONCLUSION

La diminution de l amplitude des reactions postrotatoires observee apres l operation n est pas due a la perte de perilymphe provoquée *par l ouverture de la capsule labyrinthique* En effet chez 10 grenouilles aveugles nous avons ouvert la capsule labyrinthique des deux cotes et aspire la perilymphe sans toucher au labyrinthe membraneux ni a son innervation Les animaux observes avant l operation et le lendemain de l intervention n ont montre aucune modification de l amplitude de leurs reactions postrotatoires La diminution de l amplitude des reactions rotatoires n est pas due non plus a une lésion des nerfs ampullaires des canaux semi circulaires horizontaux En effet la section des nerfs ampullaires des canaux verticaux antérieurs est une operation simple qui ne touche ni l ampoule ni le nerf ampullaire des canaux horizontaux Par ailleurs les reactions postrotatoires dirigees vers la droite et vers la gauche avaient une amplitude egale si des fibres de l un des nerfs ampullaires des canaux horizontaux avaient éte lesees les reactions auraient éte dissymétriques si des fibres des nerfs ampullaires des deux canaux horizontaux avaient éte lésees il est hautement improbable que la lesion ait ete identique des deux cotes Enfin aucune des grenouilles operees ne presentait de mouvement de manege lors d un deplacement et dans l eau notamment les animaux nageaient en ligne droite

On peut ainsi conclure de ces expériences que l elimination fonctionnelle des deux canaux verticaux anterieurs dans un peu plus de la moitie des cas (16 sur 30) et l élimination fonctionnelle d un seul canal vertical antérieur dans un peu moins de la moitie des cas *(23 sur 60) provoquent une diminution* des reactions dues à la stimulation des canaux semi circulaires horizontaux Les canaux verticaux anterieurs modifient donc dans le sens d une facilitation les reactions dues à la stimulation des canaux horizontaux (facilitation nulle a la limite)

Plusieurs hypothèses sont susceptibles de pouvoir expliquer ces resultats

1) Les influx en provenance du canal horizontal et du canal vertical antérieur convergent au niveau des motoneurones de la moelle epinière (Fig *4A*)

2) Les influx en provenance du canal horizontal et du canal vertical anterieur conver

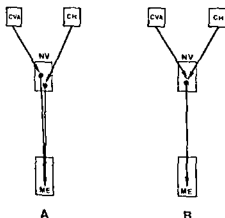


Fig 4 Representation schematique de deux hypothèses relatives a l influence du canal vertical antérieur (*CVA*) sur les réactions dues a la stimulation du canal horizontal (*CH*) *NV* noyaux vestibulaires *ME* moelle épinière (A) les influx en provenance du CH et du CVA se projettent en des endroits differents des noyaux vestibulaires les influx issus de ces centres convergent au niveau de la moelle épinière (B) les influx en provenance du CH et du CVA convergent au niveau des noyaux vestibulaires

gent au niveau des noyaux vestibulaires (Fig 4*B*)

3) Il existe, au niveau de l appareil vestibulaire, des connexions entre le canal vertical anterieur et le canal horizontal connexions qui font que l elimination fonctionnelle du canal vertical anterieur modifie le fonctionnement du canal horizontal (Fig 5)

Les deux premières hypotheses paraissent tout à fait vraisemblables et nous les avons discutees dans un travail recent (Caston, 1975) Cependant, elles n ont pas ete testees experimentalement chez la Grenouille Par contre, la troisieme hypothese a fait l objet d une etude experimentale et les resultats de cette etude sont en grande partie publies Nous rappellerons brievement ici les conclusions auxquelles nous sommes parvenus (Fig 5) Les recepteurs de l appareil vestibulaire en particulier le canal vertical anterieur et le canal horizontal, sont en relation les uns avec les autres par deux systemes distincts

1) par le systeme vestibulaire efferent les fibres vestibulaires efferentes dont l'activite est entretenue par des afferences vestibulaires contralaterales exercent une influence inhibitrice sur l activité vestibulaire afferente alors que les fibres vestibulaires efferentes dont l activite est entretenue par des afferences vestibulaires ipsilaterales sont probablement facilitatrices (Gribenski & Caston, 1975, 1976) Par cette voie, le canal vertical anterieur controlateral et le canal vertical anterieur ipsilateral exerceraient des influences respectivement inhibitrice et facilitatrice sur l activite afferente provenant du canal horizontal

2) par le systeme des fibres recepteur recepteur il existe, au sein de l'appareil vestibulaire peripherique, des fibres qui, sans passer par l encephale, etablissent des interconnexions entre les recepteurs vestibulaires, fibres que nous avons appelees « recepteur-recepteur » (Caston, 1972, Gribenski & Caston, 1974) Les fibres recepteur recepteur qui ont ete étudiees sont inhibitrices (Gribenski & Caston, 1975, Caston & Gribenski, 1975) Par cette voie, le canal vertical anterieur exerce une influence inhibitrice sur l activite afferente provenant du canal horizontal ipsilateral

Il semble donc que, sans rejeter les deux premieres hypotheses, les modifications de l amplitude des reactions rotatoires observees apres section des nerfs des canaux verticaux anterieurs peuvent s expliquer, au moins en partie par l existence de relations fonctionnelles entre le canal vertical anterieur et le canal horizontal Notons que des interrela

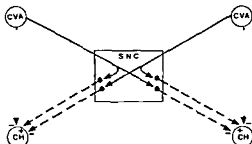


Fig 5 Representation schematique des connexions entre le canal vertical anterieur (*CVA*) et le canal horizontal (*CH*) *SNC* système nerveux central + excitation − inhibition Trait plein fibres afferentes tirets fibres efferentes pointillés fibres recepteur recepteur (les fibres recepteur recepteur ont ete representées schematiquement sous la forme de liaisons directes entre l recepteurs vestibulaires)

tions ont ete mises en evidence entre l utricule et le saccule chez le Chat (Fluur, 1970), entre l'utricule et le canal horizontal chez le Chat (Fluur & Siegborn, 1973*a b c*) et chez l Homme (Fluur, 1973), entre l utricule et les canaux semi-circulaires verticaux chez le Chat (Fluur & Siegborn, 1974*a*, *b*)

SUMMARY

We have studied the vestibular postrotatory reactions (reactions elicited by the stimulation of the horizontal semicircular canals) in the frog blinded by section of optic nerves before and after section of the ampullary nerves of the vertical anterior semicircular canals (VAC) 90 frogs have been studied In 30 frogs the ampullary nerves of the two VAC have been cut in 60 animals either the ampullary nerve of the right VAC or of the left VAC has been cut Both after section of the ampullary nerves of the two VAC and after section of the ampullary nerve of one VAC the postrotatory reactions were decreased in about the half of the animals The *decrease of the postrotatory reactions is not due to a* lesion of the ampullary nerves of the horizontal semicircular canals and it may be explained by the existence of functional connections between the VAC and the horizontal canal

ZUSAMMENFASSUNG

Bei dem durch Schnitt der optischen Nerven erblindeter Frosch wurden die postrotatorischen Vestibularreaktionen studiert d e zu der Reizung der horizontalen semizircularen Kanale (HK) vor und nach dem Schnitt der liegenden lotrechten Kanale (VLK) gebuhrt sind 90 wurden studiert Bei 30 Froschen sind die Nerven der beiden VLK geschnitten bei 30 anderen ist die Nerv des linken VLK geschnitten und bei der letzten 30 ist die Nerv des rechten VLK geschnitten Nach dem Schnitt der Nervi ampullari beiden VLK so wie nach dem Schnitt einer der beiden Nervi ampullari wurden die postrotatorischen Reaktionen in der Halfte der Falle geschwachtet Diese Verminderung ist nicht einer Verletzung der Nervi ampullari der HK gebuhrend und man kann sie durch das Vorhandensein funktioneller Beziehungen zwischen die VLK und die HK erklaren

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POSTURAL EQUILIBRIUM FOLLOWING EXPOSURE TO WEIGHTLESS SPACE FLIGHT

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Abstract Postural equilibrium performance by the Skylab 1/2 3 and 4 crewmen following exposure to weightlessness of 28 59 and 84 days respectively was evaluated using a modified version of a quantitative ataxia test developed by Graybiel and Fregly Performance for this test was measured under two sets of conditions In the first the crewman was required to maintain postural equilibrium on narrow metal rails (or floor) with his eyes open In the second condition he attempted to balance with his eyes closed A comparison of the preflight and postflight data indicated moderate postflight decrements in postural equilibrium in three of the crewmen during the eyes open test condition However in the eyes closed condition a considerable decrease in ability to maintain balance on the rails was observed postflight for all crewmen tested The magnitude of the change was most pronounced during the first postflight test day Improvement was slow however on the basis of data obtained recovery of preflight baseline levels of performance was evidently complete at the end of approximately two weeks for all crewmen The findings are explained in terms of functional alterations in the kinesthetic touch vestibular and neuromuscular sensory mechanisms induced by the prolonged absence of a normal 1-G gravitational environment

In his normal gravitational environment man has four sources of sensory information which can be used to maintain postural equilibrium vision, vestibular inputs kinesthesia, and touch Of these senses the superiority of vision as a basis of postural stability has been demonstrated by a number of investigators (Cantrell, 1963, Clark & Graybiel 1964, Edwards 1946, 1943, Passey 1950, Wapner & Witkin, 1950, Weissman & Dzendolet 1972, Witkin & Wapner, 1950 Even when other systems are non-operative, vision can be employed to maintain upright posture On the other hand, provided that the mechanoreceptors are intact, vision is not essential as evidenced by the observation that blind people have little difficulty in maintaining postural equilibrium (Edwards, 1946) There is little doubt that functional disturbances in the vestibular, kinesthetic, and tactile sensory modalities can affect postural stability People who have experienced unilateral labyrinthine or cerebellar damage will often fall to the side of the lesion (Halpern, 1954) Patients with bilateral labyrinthine disturbances, on the other hand, frequently appear to exhibit little disability in maintaining a steady posture when standing with feet together and eyes closed in the Romberg position (Birren, 1945) When the testing procedure is improved however and a sharpened Romberg is employed (Graybiel & Fregly, 1966) bilateral labyrinthine defects as well as other less dramatic vestibular disturbances do result in postural difficulties that are evident when the eyes are closed (Fregly & Graybiel, 1970) These observations suggest that in a closed loop system the sensory basis of postural stability must include inputs from kinesthetic, pressure, and touch receptors, as well as visual and vestibular inputs (Graybiel, 1973, Howard & Templeton, 1966)

That exposure to the dramatically altered environment encountered during weightless

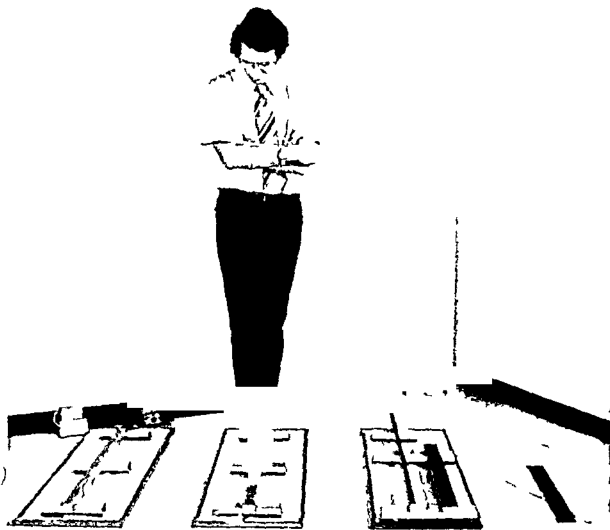


Fig 1 Illustration of postural equilibrium test rails and a subject demonstrating the required test posture

space flight may affect postural stability has been under investigation by our laboratories beginning with the Apollo 16 mission Although complete data are not available from Apollo 17 preflight and postflight testing of the Apollo 16 crewmen indicated some decrement in postural equilibrium three days following recovery when the crewmen were tested with their eyes closed (Homick & Miller 1975) Using a measurement procedure referred to as a stabilography investigators in the Soviet Union have reported that the crewmen of the 18-day Soyuz 9 mission manifested difficulty in maintaining a stable vertical posture which did not normalize until ten days after the flight The greatest disturbances were measured during an eyes closed test condition (Kakurin 1971)

On the basis of these combined observations it was hypothesized that with prolonged

exposure to a weightless environment, those sensory systems, with the possible exception of vision, necessary for the maintenance of postural stability, will undergo some changes Further, these changes are most likely originally peripheral, and involve the modification of inputs from the receptors serving kinesthesia, touch, pressure, and otolith function As exposure is prolonged, habituation responses occur at a central level in the nervous system which constitute learning in a new environment When the environment is again changed from weightlessness to a 1-G reverence, ataxia and postural instability will be manifested as the result of the neural reorganization that has occurred in weightlessness

The specific objective of this investigation was to assess the postural equilibrium of the Skylab astronauts following their return to a 1-G environment and to suggest possible mechanisms involved in any measured changes

METHOD

Postural equilibrium was tested by a modified and shortened version of a standard laboratory method developed by Graybiel & Fregly (1966) Metal rails of four widths 1 90 3 17, 4 45 and 5 72 cm (0 75 1 25 1 75 and 2 25 in), provided the foot support for the crewmen during the preflight and postflight tests In addition, rail widths of 1 27 and 2 54 cm (0.5 and 1 0 in) were available for preflight testing only A tape approximately 10 16 cm wide (4 0 in) and 68 5 cm long (27 0 in) served as a foot-guide alignment when the crewmen were required to stand on the floor Each crewmen was fitted with military type shoes for this test, both preflight and postflight to rule out differences in footwear as a variable in intrasubject and intersubject comparisons

The test rails and required body posture are illustrated in Fig 1 Time which was the performance measure of balance began when the crewman, while standing on the prescribed support with his feet in a tandem heel to toe arrangement, folded his arms His eyes remained open in the first test series In the second series, the time measurement was initiated after the crewman attained a balanced position and closed his eyes During initial preflight testing, several practice trials were allowed on representative rails until the crewman demonstrated full knowledge of the test procedure and reasonable confidence in his approach to this balancing task

During the test session the initial rail width for testing with eyes open was typically 3 17 cm (1 25 in) Three test trials with a maximum required duration of 50 sec each were given If the time limit was reached in the first two trials, a third was not performed, and a perfect score of 100 sec was recorded for the initial support width If the crewman failed to obtain a perfect score, the two largest time values for the three trials were summed to obtain the final score The choice of the second rail width depended upon the crewman's performance on the initial support width If his score was greater than or equal to 80 sec, the next smaller support width was used, if his score was less than 80 sec, the next larger support width was used Testing on a third rail size was required when both of the two previous support width scores fell either above or below the 80-sec performance level Testing with eyes closed followed the same procedures except that a larger rail support 5 72 cm (2 25 in), was typically used initially Eyes closed testing always followed testing with eyes open The time required to perform the entire test was approximately 18 min All tests were conducted with normal laboratory illumination

Three preflight baseline tests were performed on each of the SL-1/2, SL-3, and SL-4 crewmen approximately six months prior to their space flights These postural equilibrium tests were part of a comprehensive battery of vestibular tests completed by each of the crewmen at the Naval Aerospace Medical Research Laboratory

Tests following the 28-day, SL-1/2

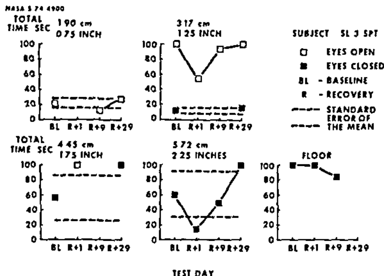


Fig 2 Postural equilibrium test performance for the Skylab-3 scientists pilot (*SPT*) The abscissa for each rail size shown indicates the days on which testing occurred including a mean baseline (*BL*) value The ordinates show total time on the rails where total time is the sum of the best two of three trials Data obtained with eyes open and eyes closed are indicated by open squares and closed squares respectively The dashed lines represent values for the standard error of the mean

were limited to balancing with eyes open and eyes closed while standing on the floor only These tests were conducted during the first (R+o) and second (R+1) day following splashdown Postflight tests on the SL-3 scientists pilot (SPT) and pilot (PLT) were conducted at 3+1, 4+9, and R+29 days following termination of their 59-day mission The SL-3 com- ' ı (CDR) was excluded from all postflight testing because of an acute back muscle strain acquired on R+o Postflight tests on each of the SL-4 crewmen were conducted on R+1, R+4, R+11, and R+31 The SL-4 flight was 84 days in duration With both of the latter two crews, the R+1 tests were conducted onboard the recovery ship which was tied to the dock and, therefore, provided a stable platform All subsequent postflight tests were conducted at the Johnson Space Center

RESULTS

Postural equilibrium tests

Preflight data obtained on these crewmen indicated that they were all well within the range of postural equilibrium performance typically exhibited by healthy aviator-type subjects

The limited postflight data collected on the SL-1/2 crewmen indicated that they all experienced considerable difficulty with standing on the floor during the eyes closed tests condition They had no trouble, however, in meeting the performance criterion when permitted the use of visual cues In considering the significance of these data, it must be remembered that the tests were performed on a moving ship

Data obtained pre- and post flight on the SL-3 SPT and PLT and the SL-4 CDR, PLT and SPT are presented in Figs 2–6, respectively In these figures, eyes open and eyes closed postural equilibrium performance on each of the rail sizes used, plus the floor is plotted as a function of test day The baseline data point shown, against which the postflight data are compared, is the mean of the preflight data for that condition The standard error of the mean was selected as a descriptor of the variance observed in the baseline data and is represented by dashed lines Approximately 50% of those cases where no variance

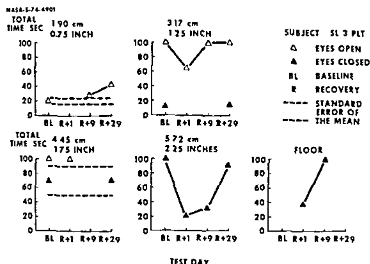


Fig 3 Postural equilibrium test performance for the Skylab-3 pilot (*PLT*) The parameters are the same as those described for Fig 2

is indicated are the results of having only a single data point on the rail size in question, otherwise, the standard error of the mean is less than one

Visual inspection of Figs 2 and 3 indicates that the SL-3 SPT and PLT showed a decrease of approximately the same magnitude in eyes open postural equilibrium performance when tested on R+1 However a more pronounced decrement in ability to maintain an upright posture was observed in the eyes closed test condition This change was more evident in the PLT and is clearly demonstrated by the 5 72 cm (2 25 in) rail size data seen Fig 3 Indeed without the aid of vision on R+1, the PLT experienced considerable difficulty even when attempting to stand on the floor, a condition he was never confronted with preflight because of his excellent balance on the 4 45 cm (1 75 in) and 5 72 cm (2 25 in) rail sizes Com

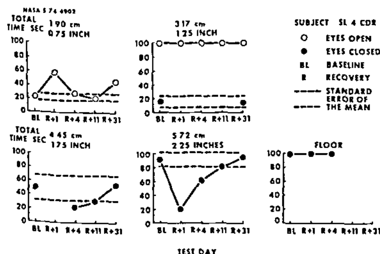


Fig 4 Postural equilibrium test performance for the Skylab-4 commander (*CDR*) The parameters are the same as those described for Fig 2

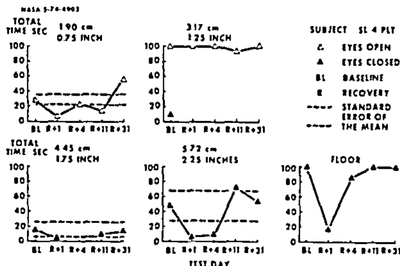


Fig 5 Postural equilibrium test performance for the Skylab-4 PLT The parameters are the same as those described for Fig 2

plete recovery to preflight levels of performance did occur in both the eyes open and eyes closed conditions for both of these crewmen However, the rate of recovery for the PLT was apparently slower as evidenced by his relatively poor score on the 5 72 cm (2 25 in) rail on R+9

In contrast to the SL 3 crewmen the SL-4 CDR and PLT demonstrated no decrease in their postflight eyes open postural equilibrium , measured by this procedure (Figs 4 and 5) ,, did however show a very large deficit in ability to balance with eyes closed In the case of the CDR this postflight change is clearly indicated on R+1 with the 5 72 cm (2 25 in) wide rail Also it can be seen that on R+1 he was almost unable to maintain the required vertical posture while standing on the floor with his eyes closed Improvement was evident on R+4 and the data obtained on R+11 indicate that both of these crewmen had regained their preflight level of ability on the eyes closed portion of this task

Data obtained on the SL-4 SPT are presented in Fig 6 It can be seen that like the SL-3 crewmen the SL-4 SPT experienced a postflight decrease in ability to maintain postural equilibrium in both the eyes open and closed test conditions The magnitude of change was much greater without vision At R+4 this change was still very evident but by R+11, this crewman's ability to balance on the test rails had returned to baseline proficiency

Subjective reports and observations

The postflight decrease in postural stability demonstrated with the rail tests are supported by observations and subjective reports by the crewmen

Although all of the Skylab crewmen were able to walk with minimal or no assistance immediately after existing the command module they did so with noticeable difficulty During this initial postflight period on the recovery ship, they tended to use a wide stanced shuffling gait with the upper torso bent slightly forward With each passing hour back in the 1-G environment they gained confidence and proficiency in their ability to walk about unaided By the end of the first recovery day all of the crewmen showed considerably improved ambulatory performance and by the time they disembarked the recovery ship (R+2) they manifested few noticeable signs of ataxia or postural instability

During the first several days following splashdown and especially on R+0 all of the crewmen reported that the simple act of walk

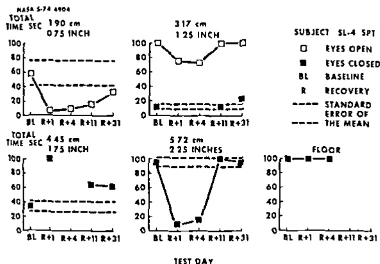


Fig 6 Postural equilibrium test performance score for the Skylab-4 SPT The parameters are the same as those described for Fig 2

ing required a conscious effort The SL-3 CDR, for example, reported that when he stepped forward, he had a feeling that he was also moving sideways Nearly all of the crewmen reported that they had to be especially careful when walking around corners because they had a tendency to fall to the outside This problem was described by a few of the crewmen as a sensation of forced lateral movement

Related to these subtle disturbances in postural stability was the report by all of the crewmen that rapid head movements produced a sensation of mild vertigo This sensation could be effectively controlled by holding the head steady Several of the crewmen including the SL-r CDR and PLT indicated a particular need to hold their head steady while attempting to balance on the test rails Any slight head movement, especially during the eyes closed test condition would induce the vertigo sensation and cause them to lose balance The movement induced vertigo diminished gradually and in most cases was gone within three to four days following splashdown, however the SL-4 PLT reported that he occasionally experienced mild vertigo with rapid head turns as late as R+11 It is also of interest to note that on R+1 and R+4, the SL-4 PLT reported experiencing a "wide deadband" when attempting to balance on the test rails with his eyes closed In other words, he was unable to accurately sense small displacements of either his head or body

Because the postflight test intervals were infrequent and not at the same times for each crew, the time course to complete recovery cannot be clearly specified However, on the basis of observations and data obtained, it appears that the Skylab crewmen required up to ten days to regain their normal postural stability These results are in close agreement with the Soyuz 9 postflight postural stability findings reported from the U S S R

DISCUSSION

The results from the present study provide evidence that postural stability can be affected by prolonged periods of exposure to weightlessness Support for the hypothesis that central neural reorganization occurs in response to environmental change is obtained when the postflight decrease in stability on the rails and the time course to recovery is compared with performance preflight

The adaptive changes may occur and contribute to disturbances of equilibrium following

exposure to a weightless environment is reasonable from a physiological point of view As one basis of postural stability vision can expect to undergo little change However the vestibular apparatus (particularly otolith input) kinesthesia and touch will be those sensory systems most affected by exposure to zero g

Subgravity levels can be experienced in parabolic flight free fall and short jumps Water immersion and sensory deprivation procedures minimize stimulation of kinesthetic and touch receptor systems without lifting the gravitational load on the otolith receptors It is only in space flight that prolonged periods of weightlessness can be achieved During these periods kinesthetic and touch stimulation (as maintained with contact to the earth through the feet and legs) is reduced and otolith input is considerably modified Static otolithinput cannot in this latter situation provide information for spatial orientation (spacecraft vertical) nor can kinesthesia or touch provide reliable sensations unless the crewman is in contact with a rigid surface to provide some reference point

That these sensory systems can adapt to the weightless environment is suggested by the creased ability with time for the crewmen to aneuver with decreasing difficulty In this regard physiological evidence has been obtained that suggests adaptation toward the norm in the frog s otolith system following four to five days exposure to weightlessness (Gualtieratti 1972) It is also possible that adaptation in weightlessness of the sensory systems basic for postural stability is similar to the changes experienced in other unusual inertial force environments such as prolonged exposure to slow rotating rooms and movements encountered on ships

If this is the case then several mechanisms could be proposed to account for the changes occurring as a result of exposure to weightlessness First a central nervous system pattern center concept (Groen 1961) could be postulated to help understand the possible mechanisms encountered in the habituation process For example following insertion into orbit the crewmen may experience difficulty in maneuvering and find orientation to be a problem After four to five days movement from one area of the vehicle to another would become somewhat easier Fine motor control to determine displacement would be established Adaptation in the postural mechanico-motor system would have occurred

On the basis of the postulated pattern center the radical environmental change encountered in transitioning from 1 G to zero-G would result in vastly different inputs from the otolith kinesthetic and touch receptors These altered inputs would then be sent to their corresponding centers and these in turn relayed to the pattern center where a copy of the appropriate movement was stored progressively over time Once an adequate memory of the pattern is built up the pattern center would take over movement and automatic balance control Further under control of peripheral inputs from the otolith kinesthetic and touch receptors relaying the actual movement the center would permit anticipation of the coming movement Return to a 1-G environment would result in a recurrence of difficulty both in locomotion and postural equilibrium Habituation to a gravity reference would begin almost immediately and a new effective pattern in the pattern center would be established

A second mechanism could possibly be responsible for the changes noted in postural stability Biosterometric analysis of body form indicated that the crewmen experienced a measurable postflight reduction in body tissue volume part of which was muscle tissue (Whittle et al 1974) A significant percentage of the total volume loss noted was in the thighs and calfs A postflight decrease in leg strength was also measured (Thornton & Rummel 1974) In the case of the SL 3 crew the average leg strength loss was approximately 20 percent As the present task required standing on the rails in a sharpened Romberg position

it is possible that the crewmen were physically incapable of completing the task due to disuse atrophy of the major weight bearing muscles

A third alternative is also possible Both a hyper Achilles tendon reflex and increased gastrocnemis muscle potential were observed postflight in the SL-3 and SL-4 crewmen (Nicogossian et al , 1974) This hyperactivity could have resulted in overreaction and overcompensation on the part of the crewmen, thus making rail performance difficult

A fourth mechanism that could be responsible for the degradation of postural stability observed postflight in the Skylab crewmen, is one which would include as contributing factors all of the possibilities mentioned Once the pattern center serving the postural, mechanico-motor system has been established in weightlessness and must begin habituation to a 1-G reference, increased reflex sensitivity may be only one aspect of the process A second aspect may be that the loss of tissue volume would contribute to a reduction in mechanical damping of leg movements For example, if we look at the pattern center serving the postural, mechanico motor system as one in which control depends on negative feedback (as the muscle spindle control system does), then it is possible for instability to occur both in locomotion and postural equilibrium The instability results because the error signal takes time to generate a corrective response This means that if no compensation for the error is programmed, the corrective signal would arrive at such a time that the leg, in this case has already moved on to a new position A second correction would be necessary which would also result in overshooting To stop this oscillation around the desired point the limb movement must be damped Pure mechanical damping is provided by the in series elastic elements in the muscles as well as the viscosity of muscle tissue and joints More tissue in the leg adds increased mechanical damping while less tissue would tend to permit undamped movements

An alternate way of viewing damping is to suggest that the reflex control system depends on an output determining both position error and the rate of change of muscle length When the system has rate-of-change information available, anticipation of the new limb position is predictable and a corrective signal can be initiated to begin a corrective adjustment (Partridge & Glaser, 1960) The hyper reflex activity observed could be a compensatory reaction generated in the mechanism responsible for programming the pattern center as a result of modified otolith input and a mechanically underdamped system

Our results tend to support this fourth hypothesized mechanism Decreased postural stability was observed in all crewmen when tested postflight Although the larger deficits were obtained when visual cues were not available, there were greater changes in postflight equilibrium in the SL-3 crew with vision than there were in the SL-4 crew Correspondingly, the SL-3 crew did not exercise to the same degree inflight as the SL-4 crew and, as a result, exhibited a greater loss in leg muscle strength and muscle tissue This suggests that vision compensated less with increasing muscle mass loss

These overall findings argue for an environment dependent memory store (pattern center) of frequently sensory inputs that is under the guidance of a combined otolith, kinesthetic, and touch system which registers the actual movement and allows for anticipation and compensation of each movement as it occurs Being environmentally dependent, such a mechanism could account for the buildup of postural responses (such as hyper reflex activity) in 0-G that would be inappropriate upon return to a 1-G reference A mechanism of this type could be applied to account for sensory physiological habituation in a variety of situations In particular, such a mechanism could provide an adequate basis for change when he acquired response patterns are not gruent with the environment.

ZUSAMMENFASSUNG

Nach 28 59 und 84 Tagen Schwerelosigkeit wurden bei den Besatzungen von Skylab 1/2 Skylab 3 und Skylab 4 mittels eines modifizierten quantitativen Ataxie Tests nach Greybiel und Fregly Gleichgewichts Untersuchungen durchgeführt Unter zwei verschiedenen Bedingungen wurde das Gleichgewichtsvermögen untersucht Zunächst mußte der Proband mit offenen Augen sein Gleichgewicht auf schmalen Metallschienen (oder auf dem Boden) halten Bei der zweiten Untersuchung mußte er versuchen sein Gleichgewicht mit geschlossenen Augen zu halten Ein Vergleich der vor und Nachflug Untersuchungsergebnisse ergab bei drei Astronauten nach dem Raumflug eine leichte Abnahme des Gleichgewichtsvermögens bei offenen Augen Bei den Gleichgewichtsuntersuchungen mit geschlossenen Augen ergab sich jedoch nach dem Raumflug bei allen 9 beteiligten Astronauten eine erhebliche Abnahme des Gleichgewichtsvermögens auf den Metallschienen Die Unterschiede waren besonders ausgeprägt am ersten Tag nach der Rückkehr vom Raumflug Die Vorflugwerte wurden nur langsam wieder erreicht Zwei Wochen nach Rückkehr vom Raumflug hatte offensichtlich das Gleichgewichtsvermögen bei allen beteiligten Astronauten denselben Grad wieder erreicht wie vor dem Raumflug Die demonstrierten Veränderungen im Gleichgewichtsvermögen finden ihre Erklärung in funktionellen Veränderungen von Mechanismen im Bereich der Kinästhetik der Berührungsempfindungen sowie des vestibulären und neuromuskulären Sinnesapparates hervorgerufen durch die lange Abwesenheit von normalen Schwerkraftbedingungen während des Raumfahrt

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HISTOPATHOLOGIC FINDINGS IN SURGICAL SPECIMENS OF ENDOLYMPHATIC SAC IN MENIERE'S DISEASE

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Abstract Two types of abnormality in the endolymphatic sac acquired at shunt surgery are presented One was deep brown pigmentation in the subepithelial connective tissue The pigment did not react to Prussian blue thus ruling out hemosiderin The other in seven out of eight specimens was the perisaccular fibrosis The fibrotic foci in four cases were assumed from the findings under ordinary illumination and polarized light microscopy to be reorganized scar tissue

Numerous morphological and experimental studies such as ablation of the endolymphatic duct (Kimura, 1967, Schuknecht et al 1968) microbiochemical analysis of the endolymph (Silverstein, 1966), and electronmicroscopic observation of pinocytotic activity in cells from the intermediate part of the endolymphatic sac (Lundquist et al 1964) have shown that the endolymphatic duct and sac exercise a resorptive function Attention is now being directed to this particular area in an attempt to elucidate the basic mechanism in the pathogenesis of clinical endolymphatic hydrops (Meniere s disease)

Shunt surgery has been used to control vertigenous symptoms of severe Meniere s disease, it also enables the acquisition of biopsy specimens We report herein the pathological changes observed in specimens acquired at epidural drainage surgery for Meniere s disease

MATERIALS AND METHODS

Materials consisted of biopsy specimens acquired at epidural drainage surgery from 8 patients who were diagnosed as having a true Meniere s disease The specimens were taken from the endolymphatic sac and fixed immediately in 10% formalin solution, dehydrated in graded series of alcohols and then embedded in paraffin Sections were stained with hematoxylin-eosin, PAS, and van Gieson, and studied under the light microscope Polarized light was used to differentiate scarred foci and necrotic tissue

RESULTS

Four of the eight endolymphatic walls appeared atrophic or fibrotic at the time of surgery (Cases 4–8) In one case (Case 1), the sac and duct were pigmented deep brown In only one case (Case 7), did the rugose portion have numerous foldings and revealed a multi lobular appearance

Histologically, in 4 cases, the epithelial linings were included in the specimens (Cases 5–8) and were composed of a flattened or low columnar epithelium In 2 cases (Cases 6, 7), the ducts were included in the specimens, lined by low columnar epithelium The ducts assumed a straight course



Fig 1 (Case 1) The rugose portion of the endolymphatic duct in a case of Meniere's disease, which assumes a loose perisaccular connective tissue Note the abundant brown pigment in the interstitial space and in the macrophages Original magnification ×448

out any recess In Case 7, there were many small split like narrow lumina, suggesting that the duct had a normal rugose structure

All but one of the biopsied perisaccular tissues (Case 1) were dense in structure and fibrous The absence of loose connective tissue was noted In 5 cases (Cases 3–7), there were foci stained pale pink with hematoxylin-eosin hich had a reduced number of nuclei of the ibrocytes and had scant vascularity The findings suggested fibrosis or necrosis Under crossed polarized light, collagen fibers in seven specimens with fibrous appearance were more intensely birefringent than the normal perisaccular tissue The relatively amorphous areas seen under ordinary illumination in 4 cases (Cases 3–5, 7) showed collagen fibrils to have a haphazard organization and varying intensity, such as is usually found in scarred tissue



Fig 2 (Case 4) Seven out of eight specimens had a dense fibrous appearance Original magnification ×160

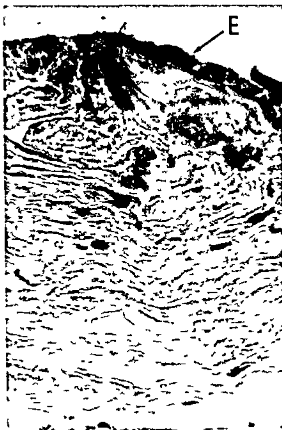


Fig 3 (Case 6) Fibril networks in the subepithelial area are somewhat disarranged Black material on the low cuboidal epithelium *(E)* is a marker made at the time of surgery for indicating the inner surface of the duct Original magnification ×320

In one specimen (Case 1), connective tissue appeared to be normal However, brown pigments were found in the subepithelial connective tissue in the interstitial space and in the macrophages The pigments did not stain with Prussian blue and could be differentiated from hemosiderin and melanin by Hueck's staining

COMMENT

Considerable attention has been directed to the pathologic changes of the rugose portion of the endolymphatic duct in Meniere's disease Our biopsied specimens revealed two kinds of pathology One was pigmentation of the duct and sac, and the other a perisaccular fibrosis

There have been two reports on brown pigment in the perisaccular tissue (Rollin, 1940, Altmann & Kornfeld, 1965) Rollin (1940) reported some observable subepithelial fibrosis and brown pigment In 1965 Altmann & Kornfeld observed coarse, brownish pigment in the macrophages of the periductal connective tissue, which was Turnbull negative They speculated on its being hemosiderin, despite its negative iron reaction As the pigmentation in the specimens reported here was observed at the time of surgery, it was not a processing artifact such as is seen with formalin pigment The specimen did not react to Prussian blue, thus ruling out hemosiderin, and was differentiated from melanin by Hueck's staining The possibility of its being lipofuscin was then considered

Of four specimens containing epithelial lining, one had a multi folding lining while the others had a straight one In the autopsy speciment, Altmann & Fowler (1943), Linsay (1942, 1944), and Altmann & Zechner (1968) also noted cases whose the endolymphatic ducts showed fewer folds in the rugose portion of endolymphatic duct in Meniere's disease

Seven out of eight specimens taken at shunt surgery for Meniere's disease in the present work appeared to be very dense in structure and fibrous, compared with the perisaccular tissue of the normal series of our temporal bone collection In the normal temporal bone, the endolymphatic sac at the border of the intradural and rugose portion is surrounded by a well vascularized, loose connective tissue Hallpike & Cairns (1938) were the first to report endolymphatic hydrops in Meniere s disease They described absence of the normal area of connective tissue around the saccus endolymphatics There have been reports of autopsy specimens since then by Rollin (1940), Altmann & Fowler (1943), Linsay (1944), Arning (1947), Cawthorne (1947), Nager (1949), Altmann & Kornfelt (1965) Altmann & Zechner (1968) in which perisaccular fibrosis was observed Shambaugh et al (1969) in a preliminary report described pathologic find



Fig 4 (Case 3) An amorphous focus in a H-E stained section under ordinary illumination (arrow) Original magnification ×160



Fig 5 (Case 3) The same area under crossed polar lights The collagen networks appear haphazard and oriented in different directions suggesting a reorganized hyalinized scar Original magnification ×320

of the surgically acquired sac at shunt surgery and observed the sac to be fibrotic

All of the previous authors concluded that the perisaccular tissue was fibrotic, only by its denser, less vascular than normal appearance There were areas in the cases in our present work where fibrocytes were scanty, vascularity was decreased, and the appearance was pale and homogeneous in hematoxylin-eosin Scar or necrosis was strongly suggested In the process of repair of a damaged tissue progressive collagenization around the proliferated fibrocytes shares the newly formed capillaries, pinches them off, and produces a relatively acellular area Therefore, observation of the collagen and its alignment may reveal tissues to have a scar

Polarized light has been suggested as a useful tool for demonstrating collagen and also for differentiating scar and necrosis (Wolman, 1975) Under crossed polarized light collagen fibrils are intensely birefringent in a parallel direction, while the necrotic tissue is not All of our seven specimens with fibrous appearance showed much more intense birefringence under polarized light than did normal perisaccular tissue In four specimens, the collagen networks were particularly haphazard sug-

gesting that reorganization had taken place Together with the findings under ordinary il lumination, the specimens with fibrotic appearance were assumed to be the foci of a hyalinized scar

ZUSAMMENFASSUNG

Zwei Arten der Anomalien die im Probestuck bei der Shunt-Operation des Saccus endolymphaticus gefunden wurden werden gezeigt Die eine war die stark braune Pigmentierung im subepithelischen Bindegewebe Das Pigment reagierte nicht mit dem Berlinerblau Dadurch wurde die Moglichkeit von Hemosidenn eliminiert Die andere die in 7 Fallen der 8 Proben gefunden wurde war die perisacculare Fibrosis Die fibrosen Foci in 4 Fällen wurden auf Basis vom mikroskopischen Befund unter der gewohnlichen Beleuchtung und dem polarisier ten Lichte auch als das re-organisierte Narbengewebe an genommen

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PERMANENT EFFECTS OF LOW FREQUENCY VIBRATION ON THE VESTIBULAR SYSTEM

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Abstract Among 49 male workers mean age 30 years who had been working in conditions of extreme noise and vibration for between 6 months and 10 years vestibular disturbances could be shown (in the form of spontaneous nystagmus lowered caloric excitability or pathology in rotatory tests) in as high as 44 9% The lesions were believed to have arisen in the peripheral vestibular organ as a consequence of the low frequency vibration

Low frequency *vibration* can cause irritation in the vestibular system The symptoms caused by vibration are mainly due to infra tones 49 male workers were studied and in about 45% of them vestibular disturbances of peripheral origin could be demonstrated

Man has a rather good absorbing mechanism against vibration of the earth or floor (Coerman et al 1960) However resonance vibration in the skull can also be caused by low frequency vibration of the air It is known that vibration especially below the frequency of 2 Hz, *causes irritation in the vestibular sys*tem, rather like sea sickness Vibration below the frequency of 10 Hz can also irritate the vestibular system These symptoms caused by vibration are mainly due to infra tones Moreover it has been shown that in many subjects ordinary noise above 130 dB can cause vertigo and even nystagmus Even lower noise levels can cause vertigo if the noise is only one sided (Ades et al 1957 Bell 1966, Kryter, 1970)

In practice, however it is not easy to distinguish between the effects of vibration and noise on the vestibular system The purpose of this study was to investigate the influence of vibration on the vestibular system In an attempt to exclude the influence of noise, only persons with normal hearing were included

MATERIAL AND METHODS

The present material consisted of two series of subjects The first series consisted of 49 male workers aged 20 to 52 (mean age 30 years) working in a room where marine diesel engines (10800 h p) are tested They had been exposed to noise and vibration for periods varying from 6 months to 10 years In their previous employment they had not been exposed to any sort of vibration None of the subjects had been exposed either before or during this *investigation to carbon monoxide benzine* trichlorethylene or other solvents

It was established that whilst the testing was going on the whole building vibrated at 2 8 to 21 5 Hz the amplitude of vibration being as weak as a mere 0 13 to 8 80 μm (Table I) The noise level in the testing room was about 118 dB (A) In this room about 18 engines are tested each year the total proving time of one engine being normally 6 to 12 hours

The control series consisted of 20 female nurses aged 19 to 24 (mean age 20 years) Pro

Table I *The vibration of the floor of the testing room when proving 10800 H P marine diesel engines at maximum power*

Measurements were made with the 1/10 octave filter

Frequency (Hz)	Amplitude (μm)
2 5	0 13
6 4	0 38
8 7	5 50
10 0	1 50
13 1	8 80
15 0	2 50
18 0	2 00
21 5	0 38

spective subjects with ear disease, head trauma as well as subjects using medicines were excluded from both series In all subjects the hearing was normal

A careful case history, and pure tone and speech audiograms were taken at the beginning of the trial Neuro-otological examinations, including ENG tests and nystagmus observations in a totally dark room with Frenzel s glasses, were made with a one month interval

Air and bone conduction audiograms were measured by the usual descending-ascending technique For each subject speech thresholds and discrimination were determined as described by Palva (1952) A Madsen OB-60 audiometer was used The rotatory tests were carried out by means of Polman s rotating chair (Polman Mod 11 e 111) which enables linear adjustment of the acceleration rate from 0 2°/sec² onwards

Silver-silverchloride electrodes 7 mm in diameter were employed The two recording electrodes were located on the skin near the temporal canthi of the lids of both eyes and the earth electrode was placed on the skin of the forehead The electrodes were fixed with adhesive tape and electrode jelly was utilized to improve the contact An a c condenser coupled amplifier time constant 2 sec, upper limit 70 Hz, was utilized for the recording All recordings were performed in darkness

The subject's head was fixed tilted 30 degrees forward from the vertical, and the eyes were closed

The threshold of acceleration was measured by the following method The subject was accelerated for 90 sec at a rate of 0 2°/sec², beginning to the right He was then decelerated at the same rate 120 seconds after stopping the same procedure was repeated, but this time in the opposite direction This procedure was continued by accelerating the subject at a rate of 0 4°/sec² and then 0 6°/sec² and finally 1°/sec² The total amplitude during the acceleration of 1°/sec² up to the final velocity of 60°/sec was also recorded and calculated

The first post rotatory nystagmus was measured after an abrupt stop (0 3 sec) Before the stop the velocity of rotation was maintained at 60°/sec during 120 sec The total amplitude of nystagmus was calculated

Any spontaneous or positional nystagmus was recorded in a dark room while the subject was lying in a supine position and in both lateral positions The nystagmus was recorded both with closed eyes and with eyes open The nystagmus was considered pathological when the speed of the slow phase exceeded 7 degrees per second

The caloric test was performed *ad modum* Hallpike with the following exceptions The irrigation time was only 30 sec The induced nystagmus was recorded for 70 sec with the subject's eyes closed At the 70th second the

Table II *Nystagmus findings and caloric reactions in subjects exposed to vibration*

	No of subjects	Per cent
Spontaneous nystagmus	10	20 4
Spontaneous nystagmus + lowered caloric excitability	2	4 1
Lowered caloric excitability	10	20 4
No nystagmus normal caloric reactions	27	55 1
Total	49	100 0

Table III *The thresholds of angular acceleration during rotatory tests*

Control series	≤0 4°/sec²	20	(100%)
Vibration series	≤0 4°/sec²	35	(71 5%)
	>0 4°/sec²	14	(28 5%)

subject was asked to open his eyes and to fix his gaze on a fixation light, situated about 2 metres away, directly in front of him For each irrigation the frequency of nystagmus and angular velocity of the slow nystagmus phase was calculated during the culmination phase, both before and after opening the eyes The result was considered as "canal paresis" (lowered excitability) when reactions before the fixation were 20% weaker in one ear than in the other The ocular fixation index was calculated according to Demanez & Ledoux (1970)

$$OFI = \frac{\text{amplitude} \times \text{frequency (eyes open)}}{\text{amplitude} \times \text{frequency (eyes closed)}} \times 100\%$$

OFI was considered normal if it was lower than 50%

The optokinetic nystagmus of the subject was investigated at 30° 40° 50° and 60°/sec to the right and to the left alternately

All these recordings were made by means of Elema Mingograph ENG The same type of electrode and the same pattern of fixating the electrodes were used as before

RESULTS

The air and bone conduction values were better than 10 dB at each frequency studied in all subjects exposed to vibration The discrimination ability of subjects was established as 100 per cent In the neuro otological examination there was no sign of any lesion of other cranial nerves

In control series there was neither spontaneous nystagmus nor abnormal caloric reactions The nystagmus findings and caloric reactions in persons exposed to vibration are shown in Table II In 10 subjects (20 4%) there was spontaneous nystagmus in all positions recorded In 2 subjects (4 1%) there was spontaneous nystagmus and lowered caloric excitability In 27 subjects (55 1%) ENG-findings were considered normal and among these subjects there was also no head-shaking nystagmus observed with Franzel's glasses in a totally dark room

The thresholds of angular acceleration are shown for both series in Table III In control series the thresholds in both directions (right and left) were equal to or less than 0 4°/s² In 35 subjects (71.5%) exposed to vibration the thresholds were the same as in the control series but in 14 (28 5%) the thresholds were more than 0 4°/s². With the method and equipment adopted in this laboratory the limit of normal threshold is 0 4°/s² (Virolainen, 1972)

The total amplitudes of the nystagmic slow phase during acceleration at a rate of one degree/s², from an angular velocity of 0°/sec to 60°/sec in both series, are shown in Table IV In the control series the mean values both to the right (106°) and to the left (103°) are statistically significantly higher than the corresponding mean values of subjects exposed to vibration (to the right 86° and to the left 72°)

The mean total (summed) amplitudes of the

Table IV *The total amplitude of the slow phase of the nystagmus on accelerating at a rate of one degree/sec² from a speed of 0°/sec to 60°/sec Comparison between control series and the subjects exposed to vibration*

Mean values are expressed in degrees

	Mean	S D	*T*	*P*
Acceleration to the right				
Control series	106	42	2 115	0 05
Vibration series	86	39		
Acceleration to the left				
Control series	103	43	3 729	0 001
Vibration series	72	32		

Table V *Mean total amplitudes of the first post-rotatory nystagmus to the right and left after an abrupt stop from a speed of 60°/sec Comparison between control series and the subjects who have been exposed to vibration*
Mean values expressed in degrees

	Mean	S D	*T*	*P*
First post rotatory nystagmus to the right				
Control series	113	45	3 613	0 001
Vibration series	77	39		
First post rotatory nystagmus to the left				
Control series	117	49	1 335	–
Vibration series	101	49		

slow phase of the first post rotatory nystagmus to the right and left after an abrupt stop from a velocity of 60°/sec are shown in Table V In the control series the mean total amplitude of right-beating nystagmus was 113° which, statistically, is significantly higher (p<0 001) than in the vibration series where it was 77° The mean total amplitude of left-beating nystagmus was in the control series 117°, and in the vibration series 101° The difference is not statistically significant

DISCUSSION

The results show clearly that many subjects exposed to vibration have spontaneous nystagmus (20 4%), lowered caloric excitability (20 4%) or both (4 2%) but no measurable hearing loss in the pure tone audiogram or lowered discrimination ability in the speech audiogram

The thresholds of nystagmus during angular acceleration are higher in subjects exposed to vibration The mean total amplitudes of nystagmus during acceleration were correspondingly lower than in the control series The mean total amplitudes of the first post rotatory nystagmus after an abrupt stop were lower in subjects exposed to vibration than in normal controls

There were no disturbances of optokinetic nystagmus The ocular fixation index (OFI) (Demanez & Ledoux, 1970) was normal in caloric tests Thus the pathological findings can be localized to the peripheral vestibular system of the labyrinth It is often impossible to differentiate between the damage caused by vibration and noise Loud noise can cause general stress reactions According to Janson (1969), vasoconstriction of the small blood vessels of the extremities arises and becomes progressively stronger with increasing sound intensity Coupled with this constriction there are changes in the arterial blood pressure (Shatalov et al , 1962, Taccola et al , 1963) Distribution of the capillaries in the end organs of the vestibular apparatus is far more developed than in the organ of Corti The activity of the protein and energy metabolism correlates with the different degrees of vascularization in the labyrinth (Schnieder, 1975)

Disturbances of equilibrium are believed to be due to noise directly stimulating the vestibular organ whose receptors are part of the inner ear structure (McCabe & Lawrence, 1958)

The control series was smaller than the series exposed to vibration and there were also only female subjects in the control series The mean age of the control series was lower than the mean age of the series exposed to vibration However the threshold of nystagmus during acceleration does not increase significantly with increasing age (Virolainen & Aantaa, 1976) or differ between sexes (Decher 1967)

Results of the present investigation indicate that low frequency vibration can be one cause of damage to the vestibular function Noise too could be the cause in the present series but all these subjects had normal hearing and there is hardly any reason for assuming that the labyrinth is more sensitive to noise than the organ of Corti It should also be noted that all subjects exposed to vibration used ear plugs when working in noisy conditions, which reduced the effects of noise but not the effects

of vibration. Vibration probably damages the labyrinth by direct mechanical trauma, other possible causative mechanisms being a circulatory disturbance in the energy metabolism of the vestibular organ Further studies are needed to resolve this problem

ZUSAMMENFASSUNG

Bei 49 männlichen Arbeitern, Durchschnittsalter 30 Jahre, die in sehr geräuschvoller und vibrierender Umgebung 6 Monate bis 10 Jahren gearbeitet hatten, konnten vestibuläre Störungen in 44,9% festgestellt werden, und zwar als spontaner Nystagmus, herabgesetzte kalorische Erregbarkeit oder Pathologie bei rotatorischen Tests Die Läsion wurde im peripherischen Vestibularorgan und die Ursache als niederfrequentes Vibrieren vermutet

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EWALD'S SECOND LAW RE-EVALUATED

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Abstract Patients and experimental animals (cats) with one functioning horizontal semicircular canal were tested with precise rotatory stimuli Nystagmus responses were quantified with EOG and a laboratory digital computer After large magnitude stimuli there was a statistically significant difference between the maximum slow component velocity of nystagmus induced by ampullopetal endolymph flow and that induced by ampullofugal endolymph flow in all patients and cats

Ewald's studies in the 1890's on endolymph flow in the semicircular canals of pigeons provided the framework for Barany's pioneering clinical vestibular investigations in the first decade of this century (Ewald, 1892, Barany, 1910) Two of Ewald's observations were so universally accepted that they became known as Ewald's first and second "laws The first law which related the direction of endolymph flow to the direction of the slow phase of nystagmus has remained unchallenged while the second law has been controversial The second law states that ampullopetal endolymph flow in the lateral semicircular canal results in a greater nystagmus response than ampullofugal endolymph flow The reverse holds true for the vertical canals This law was based on the observation that a sudden compression of the membranous horizontal canal by Ewald's "pneumatic hammer" resulted in a greater nystagmus response than did release of the compression Ewald was also the first investigator to attribute a resting tone to the semicircular canal cristae and to postulate that ampullopetal endolymph flow in the lateral semicircular canals increased this tone (*Reiz*) while ampullofugal flow reduced the resting tone (*Hemmung*)

It was not until the work of Lowenstein and co-workers (Lowenstein, 1937 1955, Löwenstein & Sand, 1940), however, that the significance of labyrinthine tone was fully appreciated These investigators demonstrated a spontaneous firing rate at rest in first-order semicircular canal neurons of the ray and dogfish that was modulated by physiologic stimuli Within a certain dynamic range, the increase or decrease in action potentials per second was directly proportional to the magnitude of head acceleration These data along with similar subsequent experimental data from frogs (Ledoux, 1958), cats (Adrian 1943) pigeons (van Eyck, 1955), and guinea pigs (Trincker, 1957), raised questions about Ewald's second law, since they suggested that peripheral excitation and inhibition from ampullopetal and ampullofugal endolymph flow were symmetrical Further, clinical studies in patients with loss of vestibular function on one

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side (either secondary to end organ or vestibular nerve damage) seemed to show symmetric nystagmus responses to clockwise and counterclockwise rotatory stimuli (Stahle 1958) For this reason rotatory tests were largely abandoned in routine clinical vestibular examinations and several investigators suggested that Ewald s second law was incorrect (Dohlman 1961 Hallpike 1961)

More recently Goldberg & Fernández found that first order neurons from the horizontal semicircular canal ampulla of squirrel monkeys had a resting firing rate (average 91 spikes/sec) that was increased by head rotation that induced ampullopetal movement of endolymph and decreased with ampullofugal movement but the excitation and inhibition were not symmetrical in most neurons (Goldberg & Fernández 1971 1971*a* 1971*b*) Large inhibitory accelerations commonly silenced the vestibular neurons discharge whereas large excitatory accelerations were unable to saturate the afferent neurons ability to produce repetitive action potentials These experimental findings provided a possible explanation for the discrepancies associated with Ewald s second law Nystagmus responses induced by small magnitude stimuli in patients with only one functioning labyrinth would be symmetrical because the excitation and inhibition of the remaining vestibular nerve firing rate would be roughly symmetrical Stimuli of larger magnitude however comparable perhaps to those produced by Ewald s pneumatic hammer would induce asymmetric vestibular nerve responses and thus asymmetric nystagmus responses

To further evaluate this problem we tested cats and humans with one functioning horizontal canal using a large range of precise rotatory stimuli and quantitative analyses of induced nystagmus Our findings are consistent with those of Goldberg & Fernandez (i e support Ewald s second law) and demonstrate the potential clinical usefulness of the asymmetry in response to ampullopetal and ampullofugal endolymph flow

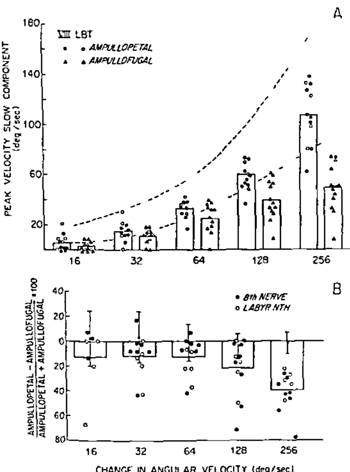
MATERIALS AND METHODS

Eleven patients with complete loss of caloric response on one side were studied In 6 the vestibular nerve was sectioned at the time of surgery for acoustic neurolemmomas and 5 were clinically diagnosed as having peripheral labyrinthine disease (3 labyrinthitis 1 vascular occlusion and 1 vestibular neuronitis) The time from documentation of complete unilateral vestibular paralysis to testing varied from 2 months to 8 years with a mean duration of 2 4 years Seven of the 11 patients at the time of testing had spontaneous vestibular nystagmus (with the eyes closed) toward the intact side

Nystagmus was induced by rotating the subjects in darkness with the head fixed in the plane of the horizontal semicircular canal All patients performed mental arithmetic throughout the testing to maintain a constant state of alertness The rotatory chair was mounted on a direct-driven platform that allowed precise control of the angular acceleration with the use of servo-controlled amplifiers Each patient was tested with a series of impulse changes in angular velocity (16 32 64 128 and 256 degrees X sec $^{-1}$ clockwise and counterclockwise) and with sinusoidal rotation at 0 05 Hz and peak velocities of 15 30 60 and 120 degrees/sec The impulse changes in angular velocity occurred with an acceleration of 14? degrees X sec $^{-2}$ Direct coupled amplifiers were used to electro-oculographically monitor eye movements Amplitude duration and velocity of the fast and slow component of nystagmus were determined by the use of a digital computer and newly developed algorithms (Sills et al 1975 Baloh et al 1975)

RESULTS

The maximum slow component velocity (SCV) of nystagmus induced by ampullopetal and ampullofugal stimulation of different magnitudes is plotted for each patient in Fig 1*A* One standard deviation about the mean re



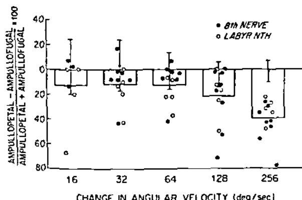


Fig 1A Peak slow component velocity (*SCV*) of nystagmus induced by impulses of acceleration resulting in ampullopetal and ampullofugal endolymph flow in 6 patients with 8th nerve section (*VIII*) and 5 patients with labyrinthine (*LBT*) disease (2 of the 8th nerve patients were tested before and after surgery and both results are included) The changes in chair velocity occurred with an acceleration of 142 degrees×sec $^{-2}$ One standard deviation about the mean response in 25 normal subjects is given for comparison (– – –) Vertical range bars represent the mean patient response for each stimulus

Fig 1B Percentage difference in peak SCV between ampullopetal and ampullofugal endolymph flow in same patients shown in *1A* At the lower magnitude of stimulation several patients did not produce measurable nystagmus accounting for the decreased number of data points The mean difference between clock wise and counter clockwise rotation in normal subjects±standard deviation is given for comparison (vertical lines) Vertical range bars represent the mean difference in patient responses

sponse in 25 normal subjects is given for comparison (dashed lines) A prominent decrease in peak SCV after the largest ampullofugal stimulus (142 degrees X sec^{-2} for 1 8 sec) is apparent Because of the large standard deviation of peak SCV in normal subjects however, the decrease in peak SCV after the largest ampullofugal stimulus was not statistically significant in all patients On the other hand the percentage difference between ampullopetal and ampullofugal stimulation was a more sensitive indicator of unilateral vestibular impairment since there was a small standard deviation in the difference between clockwise and counterclockwise rotation in normal subjects Fig 1*B* plots the percentage difference between ampullopetal and ampullofugal stimulation in each patient All patients had a significant ($p<0.05$) asymmetry of response after the largest stimulus compared to th s

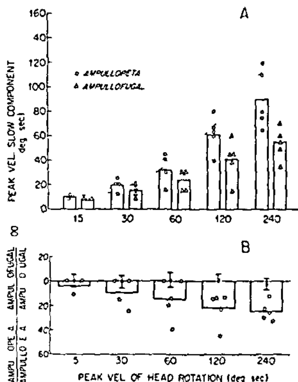


Fig 2A Peak SCV of nystagmus induced by sinusoidal rotation resulting in ampullopetal and ampullofugal endolymph flow in the left horizontal semicircular canal of 5 cats with the right horizontal semicircular canal blocked Dashed lines represent 1 standard deviation about the mean responses in 8 normal cats

Fig 2B Percentage difference in peak SCV between ampullopetal and ampullofugal endolymph flow in same cats shown in 2A The mean difference between clockwise and counterclockwise rotation in 8 normal cats is given for each stimulus (vertical lines)

25 normal subjects tested in our laboratory There was no difference between patients with 8th nerve damage and those with labyrinthine damage Two patients tested before and after surgery for acoustic neurolemmomas did not show any change in response

In 5 adult cats the left horizontal canal was blocked using the technique developed by Money & Scott (1962) None of the cats had spontaneous vestibular nystagmus after recovery from surgery Vestibular nystagmus was induced by rotating the cats in total darkness with the head fixed in the plane of the horizontal semicircular canals Only sinusoidal rotation was used at a frequency of 0 01 Hz and peak velocities of 15 30 60 120 and 240 degrees × sec $^{-1}$ The findings are similar to those from patients with only one functioning labyrinth (Fig 2A) All 5 cats had a significant ($p<0\ 05$) asymmetry in response after at least one of the two largest stimuli (Fig 2B)

CONCLUSIONS

These data suggest the following modification of Ewald s second law Ampullopetal endolymph flow in the lateral semicircular canal results in a greater nystagmus response than ampullofugal endolymph flow *after large magnitudes of head acceleration* This asymmetry in response is not simply a directional preponderance due to spontaneous nystagmus since it was present to an equal degree in patients with and without spontaneous nystagmus Even more convincing cats with one horizontal semicircular canal mechanically blocked demonstrated the same asymmetry in response to ampullopetal and ampullofugal stimulation As expected these cats did not have spontaneous nystagmus since the resting firing rate of the vestibular nerve from the blocked canal was not altered Finally the asymmetry in response was not altered with time since test results from the patient obtained 8 years after surgical resection of the vestibular nerve were similar to those from patients tested within 2 months of surgery

The magnitude of stimulus necessary to demonstrate a significant asymmetry of response in patients and cats was a sudden change in angular velocity of 256 degrees × sec $^{-1}$ (acceleration 142 degrees × sec $^{-2}$) or a maximum sinusoidal velocity of 120 degrees × sec $^{-1}$ Both of these stimuli normally induce nystagmus with a maximum SCV of at least 60 degrees × sec $^{-1}$ By comparison standard caloric testing normally results in nystagmus with a maximum SCV less than 45 degrees × sec (upper normal range in our laboratory) This explains why caloric testing has not reliably demonstrated an asymmetry in nystagmus induced by ampullopetal and ampullo-

fugal endolymph flow Goldberg & Fernández (1971*a*) found that primary vestibular neurons in the squirrel monkey varied in resting discharge rate and in the magnitude of inhibitory acceleration necessary to silence this discharge Most of the neurons that they sampled, however, were silenced by accelerations comparable to the largest magnitudes of acceleration used in this study These findings have significant clinical importance since accelerations of this magnitude for brief periods of time were well tolerated by all patients Further, by positioning each of the 3 canals in the plane of rotation it should be possible to selectively stimulate each pair and identify a single non functioning or damaged canal by the asymmetry in nystagmus response With present clinical test methodology such selective testing is impossible

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ZUSAMMENFASSUNG

Patienten und Katzen die nur einen funktionierenden horizontalen Bogengang hatten wurden durch genaue rotatorische Reizung untersucht Drehnystagmus wurde durch ENG und einen Computer quantitativ analysiert Nach hohen Reizungen war die hochste Geschwindigkeit der Langsamphase nach ampullopetalem Drehnystagmus statistisch höher als nach ampullofugaler Reizung in allen Patienten und Katzen

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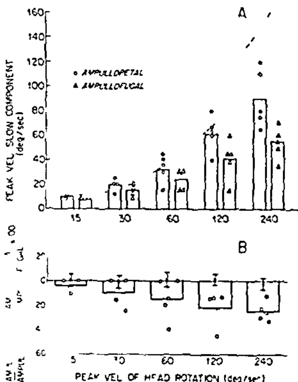


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dotally that conductive components of 15 dB are revealed by the 512 Hz Rinne and that 20 dB air–bone gaps (ABGs) are identified by the Rinne at 1 024 Hz Shambaugh (1967) claimed that a positive Rinne at 256 Hz indicates that a loss is sensorineural, with the sole exception of otitis media manifested only as a high tone conductive loss He also asserted that the number of frequencies at which the Rinne is negative relates to the size of the air–bone gap 256 Hz only for ABGs of 20–30 dB, 256 and 512 Hz if the gap is 30–45 dB, and 256, 512 and 1 024 Hz for ABGs of 45 dB or more

Hildyard et al (1963) reported survey data on children in which the Rinne at 1 024 Hz identified conductive losses as small as 15 dB Hinchcliffe & Littler (1961) cited a 1941 study by Bunch, who found negative Rinnes at 512 when the ABG was 20 dB or more In their own study, Hinchcliffe & Littler reported that the 512 Hz Rinne changed from positive to negative with an airconduction loss of 17 dB However, their data indicated that the mean loss for negative Rinnes was 32 dB Further, since only air conduction was tested, one must *assume* that the bone conduction thresh olds were normal Also, masking was not employed

Jackson & Jackson (1959) pointed out that an air conduction loss of at least 35 dB is required before the Rinne is negative Newby (1972) asserted that the Rinne is frequently not diagnostic unless there is more than a mild conductive component Martin (1972) stated that a 25 dB ABG is usually needed to over come the air-conduction advantage and thus yield a negative Rinne

Crowley & Kaufman (1966) found a change over point between positive and negative Rinnes at ABGs of about 25 dB for 256 and 512 Hz, although the Rinne was not consistently negative at these frequencies until the gaps were at least 30 dB The Rinne was reliably diagnostic in their study at 1 024 and 2 048 Hz when the ABGs were 35 dB or more Chandler (1966) found that the 512 and 1 024 Hz Rinnes were not negative even with complete occlusion of the external auditory canals By interpolation he predicted that the 512 Hz Rinne would have been negative with a 39 dB ABG, and that the 1 024 Hz Rinne would have been similarly diagnostic with a 40 dB gap

Wilson & Woods (1975) reported on the accuracy of the 256 and 512 Hz Rinnes on children They found the test to be an accurate predictor in only 40 6% of the ears One hundred percent was not reached until ABGs were at least 40 dB, and the Rinne missed 73% of the ears with conductive components of 10 to 35 dB

There is still controversy on at least several questions regarding the Rinne test How large an ABG is necessary for the Rinne to yield a negative (correctly diagnostic) result? Does this differ according to frequency? Which of the commonly available tuning forks (128–2 048 Hz) best predict(s) the presence of conductive loss? Does the size of the ABG relate to the number of frequencies at which the Rinne is negative?

These questions have received some attention in the cited reports However, of the available literature on the Rinne test, many papers are anecdotal, and the quantitative reports lack rigorously obtained data The most frequent problem has been the failure to employ masking of the non test ear in all Rinne tests A second problem relates to the omission of bone conduction audiometry, for example, when the air conduction loss was less than 10 dB in the Wilson & Woods study In this light Eagles (1961) demonstrated bone conduction thresholds in children which were better than audiometric zero This is a considerably confounding variable when the size of the ABG is important Third, the same frequencies were not used in all studies and similarly no investigation examined all five available Rinne frequencies Finally the possibility of misinterpretation of directions, especially for low tones h is been a problem This difficulty is exaggerated when the subjects are children

The current study was undert

Table I *Number (=percent) of positive and negative Rinnes and significance of differences between these, at each tuning fork frequency*

Frequency (Hz)	128	256	512	1 024	2 048
Positive (n %)	22	34	62	78	93
Negative (n %)	78	66	38	22	6
p<	001	01	02	001	001

rously investigate the above questions, which continue to exist about the Rinne test

METHOD

One hundred ears with confirmed conductive components of 73 adult males ranging in age from 21 to 64 (mean 47 8) years, served as the conductive pathology group There were 50 ears with otitis media (including a variety of types and complications) 41 with otosclerosis, eight postoperative ears (stapedectomy and fenestration) with significant residual conductive components and one ear with ossicular discontinuity The sample was not restricted to purely conductive loss, since to do so would limit the findings to a population far more circumscribed than normally seen clinically Further should the Rinne be performed in isolation there is no way to know whether the bone conduction threshold is 0 dB (or any particular level) or that it is the same in both ears

One hundred ears of 50 males with bilateral sensorineural loss of varying type and extent served as a control group They ranged in age from 20 to 66 (mean 49 3) years

Each subject received the following audiological tests (*a*) pure tone air- and bone conduction thresholds, (*b*) speech reception thresholds and discrimination, (*c*) tympanometry and stapedial reflexes All (including tuning fork) tests were accomplished in audiological examination suites exceeding ANSI standards The tests were carried out using standard procedures on two clinical audiometers (Grason Stadler 1701 and 1704) and a Grason Stadler 1720 Otoadmittance Meter, all of which were appropriately calibrated (ANSI 1969)

Aluminum alloy tuning forks of the type suggested by Sheehy et al (1971) were used Octave frequencies from 128 to 2 048 Hz were tested in random order for each ear The subjects were instructed to ignore a contralateral masking noise and to state whether the tuning fork was *clearly* louder by air- or bone conduction If a *clear-cut* difference was not evident, then the timed Rinne was performed This resulted in no cases of equivocal (air=bone) results The subjects were instructed to respond only to what was heard, and to ignore any vibrations These directions were repeated prior to every test at 128 and 256 Hz in addition to the beginning of the session

Contralateral masking was employed during all Rinne tests The masker was narrow band

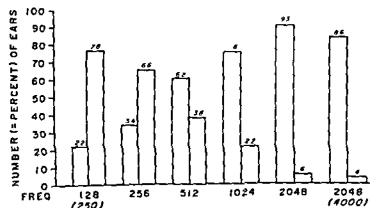


Fig 1 Number (and percent) of 100 ears with positive and negative Rinne results according to frequency Positive Rinne results are left bars and negative results are right bars

Table II *Means medians standard deviations and ranges of air–bone gaps (ABGs) for positive (+) and negative (−) Rinnes at each frequency*

Frequency (Hz)		128/250[a]	256/250	512/500	1 024/1 000	2 048/2 000	2 048/4 000
Median ABG	(+)	15	15	20	20	20	25
	(−)	40	40	40	43 5	35	30
Mean ABG	(+)	16 8	19 1	21 4	21 7	22 2	28 3
	(−)	39 0	41 8	39 9	40 9	38 3	28 8
S D	(+)	12 1	12 1	12 6	13 3	14 9	17 8
	(−)	13 4	11 7	12 3	12 0	11 7	14 9
Range	(+)	0–50	0–50	0–60	0–55	0–60	0–60
	(−)	10–60	15–60	15–60	25–55	25–55	10–45

[a] Tuning fork frequency is to left of oblique stroke and audiometric frequency to the right of the oblique

noise of appropriate mid frequency (eg , 1 000 Hz for the 1 024 Hz Rinne), delivered by a hand held earphone, and presented at an appropriate effective masking level Cases of possible overmasking were omitted from the study

RESULTS

All ears with sensorineural loss (no ABG, normal tympanogram, present stapedial reflexes, normal otoscopy, no ear complaints) demonstrated positive Rinnes at all frequencies (except for 12 ears with no response at 2 048 Hz) In several cases however, it was necessary to reinstruct the subject to respond to what was heard not felt at 128 Hz

The remainder of the data refer to the conductive ears The audiometric and impedance (admittance) findings for this group were all consistent with conductive pathologies

The number (or percent) of ears with positive and negative Rinnes at each tuning fork frequency are listed in the upper portion of Table I, and are shown graphically in Fig 1 There was no response to air- or bone conduction tuning fork stimulation at 2 048 Hz in one case The proportion of negative (correctly diagnostic) Rinnes decreased significantly with ascending frequency ($r = 1 0$ Siegel 1956) Further, there was a larger proportion of negative than positive Rinnes as 128 and 256 Hz, but a greater percentage of positive Rinnes at the higher frequencies All of the positive–negative differences were significant, although the difference at 512 Hz did not reach the 0 01 level

The relationship of Rinne results to ABG size (a direct measure of the test's precision) as each frequency is shown in Table II and in Fig 2 The Rinne at 256 Hz is compared to the ABG at 250 Hz, the Rinne at 512 Hz to the gap at 500 Hz, etc Since bone conduction thresholds are not obtained at 125 Hz, the 128 Hz Rinne is compared with the 250 Hz ABG as a compromise Analogously, the gap at 4 000 Hz is compared with the Rinne at 2 048 Hz

Except for the 2 048/4 000 Hz data[1] the median ABGs for positive Rinnes were 15–20 dB, while the median ABGs for negative Rinnes approximated 40 dB The medians and means closely approximated each other, lending further credence to the obtained measures of central tendency It is notable that there was considerable overlap in ABG size between the positive and negative cases Similarly, negative Rinnes were not obtained in any case until an ABG of at least 10 to 25 dB was obtained depending on frequency Also, ABGs of roughly 5 to 35 dB fell within ±1 S D of

[1] Ten ears had no response at 4 000 Hz Of the remaining 90 86 (95 6%) had positive Rinnes at 2 048 Hz and four (4 4%) has negative Rinnes The four negative for the 2 048/4 000 Hz combination most proba chance results

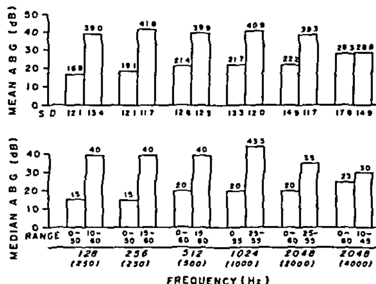


Fig 2 Mean and median air-bone gaps (ABGs) for positive and negative Rinne results according to frequency Positive results are left bars and negative results are right bars

the mean for positive Rinnes, and gaps of about 30 to 50 dB fell within ±1 S D for negative Rinnes, depending on frequency

Fig 3 shows the percentage of negative Rinnes for ABGs up to 10 dB, and for gaps of 15–20, 25–30, 35–40, 45–50, and 55–60 dB for each frequency Since The Rinne test constitutes a two alternative paradigm, 50% negative responses represents chance findings The [illegible] of negative Rinnes increased as ABGs widened except for the 2048/4000 Hz condition A clear cut off is observed at gaps of 25–30 dB for the 128 Hz data, at which point 82 6% of the Rinnes were negative Virtually all Rinnes were negative for larger ABGs, whereas smaller gaps had a negative Rinne rate that did not reach chance Gaps of 35–40 dB had 92% negative Rinnes at 256 Hz, but only 60 9% was attained at 256 Hz with ABGs of 25–30 dB Assuming a minimum identification criterion of as little as 75%, the proportion of negative Rinnes for 25–30 dB gaps at 256 Hz, and for ABGs between 35 and 50 dB at 512 Hz, must be viewed as equivocal at best The minimum 75% criterion was just attained with ABGs of 45–50 dB at 1024 Hz, *while smaller gaps did not reach even a chance level* Whereas the percentage of diagnostic Rinnes did increase with ABG size at 2048 Hz, the proportion did not reach chance even for 55–60 dB gaps For the 2048/4000 Hz condition, the Rinne was virtually never negative no matter how large the ABG

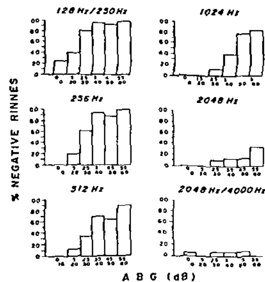


Fig 3 Percentage of negative Rinne results as a function of air-bone gap size for each test frequency

Since Shambaugh (1967) suggested that the *degree of loss relates to the number of frequencies for which an ear has negative Rinnes* the data were analysed to determine whether this held true for the current sample To accomplish this, the median ABG averaged across 250 to 4000 Hz was compared to the number of frequencies with negative Rinnes

Table III *Distribution of 100 ears according to the number of negative Rinne frequencies*

Number of negative frequencies	0	1	2	3	4	5
Number (percentage) of ears	22	11	28	19	14	6

for each ear The Spearman rank correlation (Siegel, 1956) for this was 0 943, which is just significant at the 0 01 level, suggesting that the number of frequencies with negative Rinnes is proportional to the overall extent of the conductive component

Table III shows how many ears with conductive pathologies demonstrated one negative Rinne, two negative frequencies, etc All of the ears with only one negative Rinne demonstrated this at 128 Hz Of the 28 ears with negative Rinnes at two frequencies, 128 Hz was always negative, with the second frequency being 256 Hz in 27 ears (96 4%) and 512 in one ear (3 6%) Both 128 and 256 Hz were always negative in the 19 ears with three negative Rinnes The third frequency was 512 Hz in 17 ears (89 5%) and 1 024 Hz in two ears (10 5%) When four frequencies were negative, these were always 128 through 1 024 Hz The 2 048 Hz Rinne was negative only in the six ears for which all five frequencies were negative

Three broad categories of abnormality were included in the sample, as well as one ear with ossicular discontinuity Table IV shows the percentage of ears with negative Rinnes for each group and for the sample as a whole The proportions for the subgroups were compared with those of the total sample at each frequency using chi-square tests of goodness of fit (Siegel, 1956) The significant differences are indicated on the table Analysis of the raw data, however, revealed that the ABGs were generally larger in the otosclerotic and postoperative than in the otitis media cases

DISCUSSION

The ability of the Rinne test to identify conductive loss decreased significantly as tuning fork frequency rose from 128 to 2 048 Hz Significantly more negative than positive Rinnes were obtained only at 128 and 256 Hz, while a greater proportion of erroneous results was evident at 512, 1 024 and 2 048 Hz It is noteworthy that none of the major writers suggests using 128 Hz as a Rinne frequency due to the possibility of vibrotactile rather than auditory responses to bone-conduction at this frequency DeWeese & Saunders 1973), in fact, specifically proscribed 128 Hz for this reason Further a number of sensorineural ears in the current study initially showed erroneous negative Rinnes at 128 Hz in response to tactile stimulation and had to be reinstructed in order to obtain the correct (positive) result When this occurred (as it did) with several subjects with confirmed conductive losses they too were reinstructed However it cannot be categorically stated that all of the 128 Hz negative Rinnes were truly auditory responses The possibility remains that some may have resulted from a highly (or completely) attenuated air-conduction signal

Table IV *Percentage of ears with negative Rinnes for the three subgroups and the total group of subjects with confirmed conductive pathologies*

Frequency (Hz)	128	256	512	1 024	2 048	2 048/4 000
Total group ($N=100$)	78	66	38	22	6	4
Otitis media ($N=50$)	60[a]	50[a]	22[a]	10[c]	6	4
Otosclerosis ($N=41$)	87 8	82 9[a]	56 1[b]	34 1[c]	4 9	2 4
Postoperative ($N=8$)	100[a]	75	37 5	25	0	0

Percentages marked are significantly different from the total group percentage at that frequency (*a* $p<0\ 01$ *b* $p<0\ 001$ *c* $p<0\ 02$)

combined with a tactile response to bone-conduction This may also apply to some of the 256 Hz results, although none of the subjects mentioned that vibration confused them at 256 Hz

In terms of ABG size, the data suggest that conductive components approximating 40 dB are needed for a negative Rinne to be obtained Recalling that most (±1 S D) negative Rinnes had gaps between 25 and 55 dB, while most positive Rinnes had ABGs up to 35 dB, it is evident that the Rinne lacks precision in identifying many conductive losses This was further demonstrated by the excessive overlap in ABGs (up to 60 dB) for the positive and negative Rinnes at each frequency The ABG size needed before the Rinne is able to identify even 75% of the conductive components also *indicates that the test lacks acceptable precision*

The current data are generally consistent with Wilson & Woods' (1975) results, and with Chandler s (1964) estimates In each case, an ABG of 40 dB is the most consistent estimate of central tendency for the Rinne to be negative

While the current data agree partially with those of Crowley & Kaufman (1966), there are several points of disagreement *All* 256 and 512 Hz Rinnes were negative with ABGs of 30 dB and above and all but one were negative with gaps of 35 dB or more for 1024 and 2048 Hz, in their study These findings were not evident in the current investigation Further, while they found a high proportion of negative Rinnes at 2048 Hz these were virtually absent in the current data The present results support their contention that a negative Rinne indicates an ABG of *at least* 20 dB but the data contradict their statement that a positive Rinne demonstrates that any ABG is 30 dB or less Crowley & Kaufman s suggestion that as much information of a qualitative nature is supplied by 512 Hz alone as by 256–2048 Hz is also contradicted by the present data

More frequencies showed negative Rinnes as the ABG widened However this correlation shows only that negative Rinnes and gap size are *in step* not that the number of one predicts the size of the other Also, this was a group finding for ABGs collapsed across frequency, and was not found in all cases—especially when the ABGs were different at different frequencies Thus, the individual data revealed that while more negative Rinne frequencies indicated generally wider ABGs, the absence of many negative Rinnes did not rule out a large conductive component This again suggests that the Rinne is powerful only when it is negative, but that it is deluding when positive

It may be concluded that an ABG of 25 to 40 dB, depending on frequency, is necessary for the Rinne to demonstrate the presence of conductive pathology The inability to obtain *a negative Rinne in many ears with conductive components* markedly reduces its precision as an independent diagnostic or screening test Thus, it cannot be confidently used as a criterion against which other tests can be validly evaluated While a negative Rinne is diagnostic of conductive pathology, a positive result is neither a criterion for ruling out conductive loss, nor an indicator of pure sensorineural loss, since positive Rinnes may be obtained whether the conductive mechanism is normal or not

The lower frequency tuning forks maximize the Rinne's precision however, the possibility of tactile response must be remembered Also, a fairly large number of conductive components will be missed even with low tone tuning forks especially those manifesting themselves as isolated high frequency air-bone gaps

ACKNOWLEDGEMENTS

The assistance of Dr Andrew Meyer is appreciated

ZUSAMMENFASSUNG

D e Genauigkeit des Rinne Tests mit 128–2048 Hz wurde untersucht Die Anzahl der negativen Rinne Ergebnisse fiel mit ansteigender Frequenz ab Fur 128 Hz und 256 Hz

ergaben sich große Anzahlen von positiven Rinne Ergebnissen während für höhere Frequenzen die positiven Ergebnisse überwogen dies deutet darauf hin daß der Rinne Test für Frequenzen über 256 Hz nicht zuverlässig ist Allerdings sollte man vibrotactile Responses als mögliche Fehlerquelle bei niedrigen Frequenzen betrachten Ebenfalls wird die Kluft zwischen Knochen und Luft bei höheren Frequenzen nicht durch niederfrequente Rinne Tests identifiziert Luft Knochen Klüfte von 25 bis 40 dB frequenzabhängig sind notwendig für die Genauigkeit des Rinne-Tests Luft–Knochen Klüfte von 25–30 dB für 128 Hz 35–40 dB für 256 Hz 55–60 dB für 512 Hz und 45–50 dB für 1024 Hz werden benötigt um 75% Genauigkeit zu erzielen Bei 2048 Hz besteht keinerlei Zuverlässigkeit

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DIMENSIONS OF IMPORTANCE IN RECONSTRUCTIVE MIDDLE EAR SURGERY

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Abstract A study of the space available for an ossicular prosthesis is presented Twelve temporal bones without any signs of chronic ear disease were used The measurements were made with the bone free from soft tissue mounted on a microscope cross table The different distances were registered through a stereo-microscope The width between the facial nerve canal and the promontory was found to be 2 2 mm The distance from the footplate to the tympanic sulcus plane was found to be 6 6 mm The space available for the head of a prosthesis was also investigated

For the reconstruction of a defective ossicular chain many types of autologous, homologous and alloplastic material have been used However, each of these materials has its drawbacks In an experimental study (Brånemark et al , 1976) we have shown that a titanium mould, the shape of a middle ear ossicle placed in the proximal tibia metaphyses of dogs and rabbits for about 4 months will fill with bone This bone has biological properties which ought to render it close to the ideal prosthesis Among the properties of an ossicle produced in this way is a periostal layer which acts as a biological barrier and would theoretically diminish the tendency of adhesions and anchylosis on surrounding mucosa and bone tissue On the other hand this means that the ossicle cannot be adjusted during surgery without destroying this coating Only in the free ends of the new ossicle i e where it is in contact with the stapes/footplate and the tympanic membrane/transplant can it be adjusted during surgery. In order to design a titanium mould for a prosthesis suitable for use in man, accurate information is necessary concerning the size and shape of the tympanic cavity in the region where the ossicle is to be placed

Eggston & Wolff (1947), Wolff et al (1957) and Saito et al (1971) are some of the authors who have published measurements of the middle ear, and Litton et al (1969) and Miehlke (1973) have studied the Fallopian canal However, these measurements do not concern the area in which we are interested in this special case

Barry Anson, Theodor Bast and James Donaldsson have in several papers over a number of years presented excellent studies on the size and shape of the middle ear and which are collected in their brilliant monograph *Surgical Anatomy of the Temporal Bone and Ear* (Anson & Donaldson, 1973) The stapes is well described here In 75 consecutive specimens they found the average height to be 3 26 mm measured from the under surface of the footplate (min 2 56 mm, max 3 78 mm) The width of the footplate averaged 1 41 mm (min 1 08, max 1 66 mm) and the average length of the footplate was 2 99 mm (min 2 64 mm max 3 66 mm)

The purpose of the present study was to estimate the following distances which we

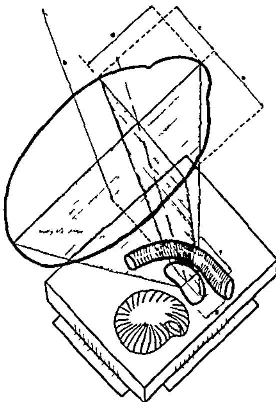


Fig 1 Schematic drawing of experimental set up Symbols b c d e see legend in Table I f 2 99 mm and g 1 41 see text

have not been able to find described earlier in a way which is useful for our purpose

(a) the width between the facial nerve and the promontory at its narrowest point

Table I *Distances of importance in reconstructiv e ear surgerv*

N=Number of bones M=Mean values in mm V=Variation coefficient=S d ×100/M a Distance facial nerve–promontory b=Distance foot plate–sulcus tympanicus c=Distance vertical central axis–anterior direction (see Fig 1) d=Distance vertical central axis–superior direction (see Fig 1) e=Distance vertical central axis–posterior direction (see Fig 1)

	N	M (mm)	V (%)
Distance a	11	2 2	13 6
Distance b	12	6 6	6 0
Distance c	12	5 3	14 0
Distance d	12	3 4	33 4
Distance e	12	3 8	16

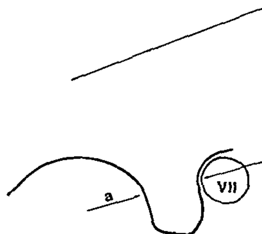


Fig 2 Schematic drawing of columella and the facial nerve/promontory area

(b) the distance from the footplate to a plane through the tympanic sulcus

(c) the position of the perpendicular projection of the mid point of the footplate on the tympanic sulcus plane (describing the space available for the head of the prosthesis)

MATERIAL AND METHODS

Twelve temporal bones were examined All of them were from adults the youngest being 58 years old and the oldest 73 years, mean age 65 According to available information none of the patients had had any trouble with their ears and when the tympanic membrane was inspected it was found normal in all temporal bones The temporal bones were processed in the following way

1 The bone was boiled in water for 1 hour,
2 soft tissue was removed from the bone,
3 tympanic membrane and ossicles were removed under microscopic preparation
4 the mucous membrane of the tympanic cavity was destroyed with 1 0 M sodium hydroxide for 15 min,
5 the bone was finally treated with 10% hydrogen peroxide for 30 min and then dried

For the measurements a stereo-microscope was used with a magnification of 40×.

A hair-cross was placed in the focal plane of one of the oculars. Horizontal movements were registered by means of a cross table equipped with nonie scales. The vertical stand of the microscope was also provided with a nonie scale. Thus movements in all three planes could be registered.

The temporal bones were mounted in such a way that the long axis of the oval window coincided with one of the axes of the cross table. This means that the short axis of the window coincided with the other axis of the cross table. The specimen was then orientated so that the plane of the tympanic sulcus corresponded to the horizontal plane (Fig. 1). All measurements were done twice so that the error of the method could be calculated.

RESULTS

The distances of importance in reconstructive surgery are listed in Table I and illustrated in Figs. 1 and 2. The width over the oval window between the facial nerve canal and the promontory was found to be 2.2 mm. This means that a prosthesis passing through this area must be thinner than 2 mm. The distance between the stapes footplate and the plane through the tympanic sulcus was found to be 6.6 mm, which is the length needed for a columella from a footplate to the tympanic membrane without any allowance for the conical shape of the drum. The measurements in the plane of the tympanic sulcus (*d, e, f* in Fig. 1) indicate the space available for the head of the prosthesis. Contact with surrounding tissue involves the risk of adhesions and anchylosis.

These dimensions of the temporal bone were measured on bones without any history or signs of chronic ear disease, which should be borne in mind when the space available for the prosthesis in the reconstructive work is estimated.

ZUSAMMENFASSUNG

Der zugängliche Raum für eine Gehörknöchelchenprothese wurde studiert. Zwölf Schläfenbeine ohne jede Zeichen von chronischer Mittelohrentzündung wurden benutzt. Die Beine, von Weichteilen gereinigt, wurden stereomikroskopisch gemessen. Der Abstand zwischen Canalis nervi facialis und Promontorium betrug 2.2 mm und die Höhe zwischen Basis stapedis und die Ebene des Sulcus anuli tympanici war 6.6 mm. Der zugängliche Raum für den oberen Teil der Prothese wurde ebenso gemessen.

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THE MUCOCILIARY ACTIVITY OF THE UPPER RESPIRATORY TRACT

I A *Method for Use in Experimental Studies on Human Material*

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Abstract A method for standardized recordings of the mucociliary activity of the mucosa in the human respiratory tract is described Nasal polyps adeno d vegetations and b opsy material from maxillary sinuses have been used for preliminary *in vitro* experiments The dependence of the mucocil ary activity on oxygen supply from the surround ng air is emphasized The influence of vary

is under way in order to elucidate the aetiology treatment and prognosis of diseases of the upper resp ratory tract

The increasing pollution of the air in towns factories and workshops especially in combination with tobacco smoking is injurious to health Harmful airborne particles such as chemical substances bacteria and viruses are rejected or locally neutralized by the defence mechanisms of the respiratory mucous membrane These mechanisms comprise chiefly (1) the mucociliary transport system (2) the immunological function of the mucosa and (3) phagocytosis

The mucociliary activity in the nose and paranasal sinuses can be studied by observation of the transportation of particles along the surface or by recording the local pattern of the undulatory movements of the blanket of mucus covering the mucosa The latter approach has recently been used in a series of *in vitro* investigations of the tracheal mucosa in the rabbit with special reference to the effect of variation of the temperature of the ambient air and of its humidity (Mercke et al 1974*a* *b* Toremalm et al 1974 Mercke 1975) However corresponding investigations on the mucociliary activity of the human mucosa in healthy and pathological conditions in order to elucidate the aetiology treatment and prognosis of diseases of the upper respiratory tract are still lacking Such functional investigations require a standardized method filling the following requirements

1 The method should be applicable to *in vitro* as well as *in vivo* conditions

2 The equipment for mucociliary activity studies should therefore be uncomplicated and portable

3 It is essential to maintain the sterility of those parts of the equipment that are applied to the patient

4 Instantaneous interpretation of the mucociliary activity should be possible

This paper describes such a method and some preliminary experimental investigations of human mucous membranes in a model of

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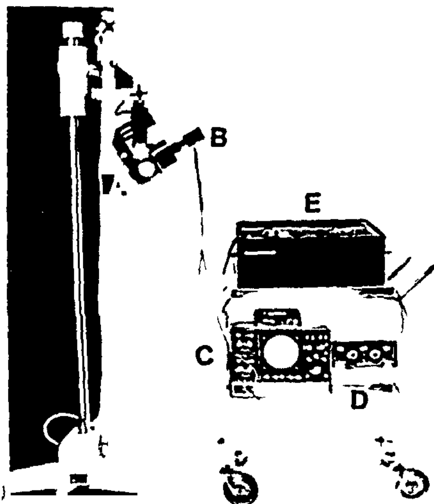


Fig. 1. Apparatus for clinical recording of the mucociliary activity in the human upper respiratory tract. Binocular operating microscope (A), photodetector replacing one of the eyepieces (B), oscilloscope (C), frequency filter (D) and ink writer (E).

the adult maxillary sinus. The apparatus is also designed for alternative use in clinical *in vivo* studies in association with operations on maxillary sinuses (Reimer & Toremalm 1977).

METHOD

The variation in the light reflected from the illuminated surface of the blanket of mucus covering the ciliated epithelium is observed through an operating microscope. Variations in light intensity are transformed by a phototransistor to electrical signals, which are recorded instantaneously by an ink writer.

Equipment

A binocular operating microscope (Zeiss OPMI I) with an objective magnification of 2× is used (Fig. 1A). The original illumination from the microscope is used. The angle between incident and reflected light is less than 20°. Light reflections from the mucosa are seen through one of the eyepieces, with a magnification of 12.5×. The other eyepiece is replaced by a phototransistor housed in a rectangular case measuring 9.5×4.5×2.5 cm (Fig. 1B). The transistor (FPT 130) is connected as a diode. The circuit diagram is shown in Fig. 2. The device is powered from a low voltage d.c. source. The phototransistor

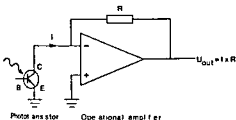


Fig 2 Circuit diagram of the photodetector

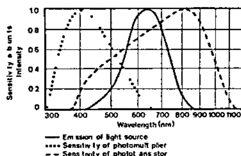


Fig 4 Emission spectrum of the light falling on mucosa (—) The spectral sensitivity of the photomultiplier ( ) and of the phototransistor (-)

is placed in the image plane of the objective The light-sensitive surface is covered by a diaphragm with a circular aperture (0 2 mm in diameter), which is placed in the optical central axis The illuminated area of the phototransistor thus corresponds to an observed circular mucosal area 0 1 mm in diameter Recordings are made by an ink writer (Elema-Schonander Mingograph 34, Fig 1 E) A frequency filter (Krone-Hite, 3550, Fig 1 D) is included before the ink-writer

Comparison between photomultiplier tubes and phototransistor for mucociliary activity studies

In the photomultiplier tube, which was used in our previous studies (Mercke et al 1974*a*), the light to be measured is projected or directed against a cathode coated with suitable light sensitive material (Fig 3, above) The photoelectric effect releases electrons which are accelerated and cast against dynodes in the photomultiplier tube, where an increasing number of electrons are released and attracted to the anode of the tube The current thereby created is then conducted through an external load resistor across which any decrease in potential is recorded The photomultiplier tube is highly sensitive and has a frequency response within the visible range from d c to 100 MHz It requires a power supply of 1 000 V and is much larger than the phototransistor

The phototransistor used in the present equipment is a semiconductive component with two p doped areas and one n doped area (Fig 3, below) A p-doped area has more positive than negative charge carriers The protons are therefore called majority charge carriers and the electrons minority charge carriers In the n-doped area the conditions

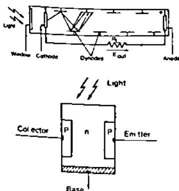


Fig 3 Above Photomultiplier principle *Below* Phototransistor principle (For details see text)

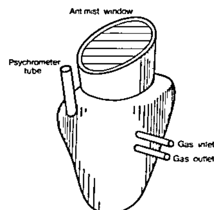


Fig 5 Model of the maxillary sinus of an adult The model is provided with an oblique glass lid for incident light and reflected light as well as an inlet and an outlet for test gases and an outlet to a micropsychrometer

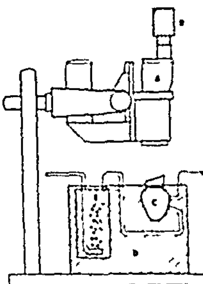


Fig. 6. The operating microscope (A) and photodetector (B) are directed at the model of the maxillary sinus (C) in a thermostatically controlled water bath (D). Air, gas or gas mixtures are supplied via a thermostatically controlled saturator (E).

are reversed. When a p-doped layer and an n-doped layer are pressed together the different charge carriers assume such positions that a balance is set up at the junction between the layers. When the p/n junction is illuminated, pairs of charge carriers are produced at the junction. This disturbs the existing balance with a change of potential as a result. A loading resistor can be inserted between the collector and the emitter. The voltage drop across the resistor is amplified and recorded. This additional loading reduces the sensitivity. Alternatively, the transistor can be connected direct to a current amplifier. This does not affect the sensitivity of the transistor. Silicon (Si) is used as a semiconductive material. The phototransistor is a small component compared with the photomultiplier tube and is therefore easier to fit to the operating microscope, but it is not so sensitive to light as the photomultiplier tube. Furthermore it is temperature dependent, but this has not been a problem in the present studies as this part has been kept in ordinary room air.

Fig. 4 illustrates one of the most important advantages of the phototransistor over the photomultiplier previously used (Mercke et al., 1974*a*). The figure shows the emission spectrum of the incident beam of light and the spectral sensitivity zones of the photomultiplier and phototransistor. The transistor matches the spectrum of the incident light more closely than does the photomultiplier and it consequently reproduces the light fluctuations more faithfully.

Application

The *in vitro* application of the method is apparent from Fig. 5 which illustrates a model of an adult human maxillary sinus made of *mouldable synthetic material (polyvinyl chloride)*. Uppermost in the illustration can be seen an oblique glass window corresponding to the anterior wall of the maxillary sinus. To permit illumination and inspection of the interior of the model, the window is kept free from condensation by a small wire loop heater. This window is hermetically sealed when closed, but can be removed to enable a 4-5 mm² specimen from an operation to be deposited on a bed of cotton wool soaked in Ringer's solution at the bottom of the model. Air or test gases are circulated through the model via the two small tubes labelled "gas inlet" and "gas outlet". A psychrometer is connected for continuous control of the gas temperature and relative humidity by means of a further tube. The entire model is submerged in a thermostatically controlled bath which ensures stable temperature and humidity of the inflowing air or gas mixture (Fig. 6).

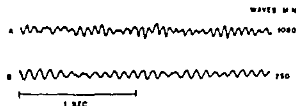


Fig. 7. The mucociliary activity on nasal polyps from two different individuals (A and B) showing a mucociliary wave frequency of 1080 and 750 waves/min respectively. Temperature 37°C and r.h. 80%.

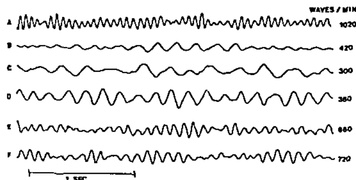


Fig 8 Ciliary activity on the surface of an adenoid vegetation in a 4 year-old patient *A B* and *C* show exposure to 100% N_2 passing slowly (about 0 1 litre per minute) through the model *D E* and *F* show how the mucociliary activity becomes normal again on supply of conditioned air Temperature 37°C and r h 80%

RESULTS

Nasal polyps were used in the first series of experiments Immediately after the operation (evulsio) they were placed in the model The temperature was kept at 37°C and the relative humidity above 80% The recordings of the mucociliary activity from two different patients are shown in Fig 7 The upper one (A) shows a mean frequency of 1 080 waves/min, while the one below (B) only shows a mean frequency of 750 waves/min The recording shown in Fig 8 refers to an epipharyngeal adenoid from an otherwise healthy child The adenoid was placed in the model immediately after the operation Curve A in Fig 8 shows the mucociliary activity at 37°C and a relative humidity of more than 80% The segments B and C were recorded in an anoxic atmosphere of moistened pure nitrogen after 3 and 5 min respectively The following segments D, E and F illustrate how the mucociliary activity successively recovered after it had been re-exposed to air (37°C, r h 80%) for 5 min

The mucociliary activity of the mucosa from a maxillary sinus is illustrated in Fig 9 The specimen had been excised from a 32-year-old man The indication for the operation was chronic sinusitis X-ray examination showed marked swelling of the mucosa The cavity contained no purulent substance but the mucosa was thickened and oedematous Microscopic examination confirmed the diagnosis of chronic nonspecific inflammation The specimen was placed in the model of the maxillary sinus after operation and kept at a temperature of 20°C and a relative humidity of more than 80% for 24 hours The upper curve in Fig 9 was recorded 30 min after the operation and the lower 24 hours later The mucociliary activity had fallen from an initial 660 waves/min to 360 waves/min

DISCUSSION

The method described is a technical improvement of that used in an earlier investigation of the mucociliary activity of the rabbit trachea under standardized temperature and humidity conditions of the ambient atmosphere (Mercke et al , 1974*a*) A photomultiplier was used in those investigations, while a phototransistor has been tested during the present experiments The difference between the two methods is described above One particular advantage of the phototransistor is that its sensitivity more accurately covers the emission spectrum of the light source (Fig 4) A practical simplification is that the transistor

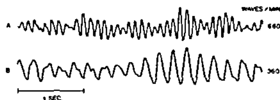


Fig 9 The mucociliary activity of a piece of mucosa excised from the maxillary sinus *A*=30 minutes after operation *B*=24 hours after the operation Temperature 20°C and r h 80%

only needs a 2×15 V d.c. power supply instead of the very high voltage power supply required previously. The entire set up is thus portable and less cumbersome and is easily transported from the laboratory to the operating theatre. It also allows recording with an angle of less than 20° between the incident and the reflected light beam. The light-sensitive part of the transistor is placed in the image plane of the objective, which gives better mechanical stability than a photomultiplier mounted on the eyepiece. The unit attached to the microscope is thus smaller, which enables the surface of the mucosa to be inspected through the second ordinary eyepiece. The phototransistor is kept at constant room temperature so that interference due to temperature variations is avoided.

Nasal polyps are usually covered with a ciliated epithelium and are easily available for clinical and experimental exposure studies. Individual differences in mucociliary activity can be recorded as shown in Fig. 7. Further studies are indicated in order to recognize possible differences in the mucociliary activity in individuals with different nasal diseases. Adenoid vegetations from human beings have also proved useful in experimental exposure studies regarding the mucociliary activity, since they are easy of access. The initial frequency of the mucociliary wave movements on human adenoids and polyps was about 1 000 waves/min, i.e. about the same as found in our previous investigations on the rabbit trachea. Pathologically modified mucous membranes representing different diseases of the respiratory tract can readily be obtained from biopsy specimens during operations, e.g. in the nose and paranasal sinuses. A piece of mucosa of 4 to 5 mm² is ample. One example is illustrated in Fig. 9. The mucosa can survive for at least 24 hours in the model. For practical reasons this experiment was made at room temperature (20°C). The frequency of the mucociliary activity was therefore already initially lower than normal (600 waves/min), which is in accordance with previous temperature exposure experiments (Mercke et al. 1974*b*).

The model of the experimental chamber has been designed so that it facilitates comparative *in vitro* studies and clinical *in vivo* studies of the mucociliary activity in maxillary sinuses. This development of the method will be further discussed in a forthcoming paper (Reimer et al., 1977).

Survival of the activity of the cilia *in vitro* requires not only suitable temperature and humidity but also a satisfactory oxygen level in the ambient air. If ciliated mucosa kept in an ideal temperature and humidity is exposed to conditioned pure nitrogen, the beating of the cilia will rapidly cease (Fig. 8). However, supply of air will soon be followed by recovery of the function of the cilia. This special problem will be investigated by a series of further experiments, which are already in progress.

ZUSAMMENFASSUNG

Beschreibung einer Methode für standardisierte Aufzeichnungen der mukoziliaren Schleimhautaktivität im humanen Luftweg. Nasenpolypen und adenoide Vegetationen sowie Biopsiematerial aus Kieferhöhlen wurden in einleitenden *in vitro*-Versuchen benutzt. Die Bedeutung der Sauerstoffzufuhr aus der umgebenden Luft für die mukoziliare Aktivität wird unterstrichen. Der Einfluß verschiedener Gasmischungen auf die mukoziliare Aktivität kann eingehend studiert werden. Die Methode ist auch für *in vivo*-Aufzeichnungen während der Luc-Caldwellschen Operation verwendbar. Eine Kombination von *in vivo*- und *in vitro*-Studien wird beabsichtigt, um die Ätiologie, die Behandlung und die Prognose bei Erkrankungen der oberen Luftwege zu klären.

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NASAL GLANDS IN NASAL ALLERGY

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Abstract. The entire nasal mucosa from a patient with nasal allergy and hay fever was studied by the whole mount method and the density of submucous glands was determined. Their density was essentially greater than in normal noses in all parts of the nose, especially on the conchae. This finding indicates that new abnormal glands form in the course of the disease, glands which also differ morphologically from the normally occurring glands. As this greatly increases the secretory capacity of the mucous membrane, there is a patho-anatomical basis for assuming that the nasal secretion in allergy is formed exclusively by the existing glands. Other sources of nasal secretion, such as exudation and transudation under normal conditions and in allergy, as well as the mode and causes of gland formation, are discussed.

Nasal secretion is greatly increased in patients with allergic diseases of the nose. This increase may manifest itself as attacks of aqueous secretion in hay fever, as chronically increased mucous secretion or as mucopurulent secretion in the event of superimposed infection of the mucosa which often attends allergic nasal disorders. The secretion may be a transudate, an inflammatory exudate, or a product of the secretory elements, viz. the epithelial goblet cells and intraepithelial serous anterior nasal glands (Bojsen-Møller, 1965), numerous small sero-mucous glands from the entire respiratory region, and lastly, serous Bowman glands from the olfactory region. It would be desirable to know the share of the various elements in secretory production in nasal disorders.

Previous Investigations

By summing up earlier findings and from his own comprehensive investigations Hansel (1930) found the following histopathological changes in allergic nasal mucosa. Thickening and hyperplasia as well as polypoid degeneration of the *epithelium*, initially with an increase in and later with a decrease in goblet cells. There would be eosinophilic, mononuclear, and lymphoid cell infiltration, oedema, and proliferation of the fibrous tissue, dilatation, thickening and compression of blood vessels *in the lamina propria*. He observed considerable variation in the pathological changes of the glands. Hyperplasia and distention of acini with secretion, degeneration and obstruction of the acini and ducts, cystic dilatation and periglandular round-cell as well as eosinophilic-cell infiltration of the superficial glands. Furthermore, there would be compression or dilatation and atrophy of the deeper glands, especially in areas with compact fibrous tissue. Brunner (1942) found a similar polymorphous appearance of the glands also in other, non-allergic hyperplastic nasal diseases. More recent investigations of allergic nasal mucosa have shown mainly an increase and hyperactivity of the glandular tissue (van Dishoeck & Meyer 1964, Puskás et al. 1969, Jahnke, 1972). By scanning electron microscopy Mygind & Bretlau (1974) observed more glandular orifices in perennial

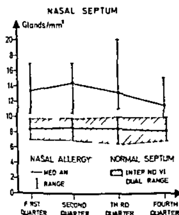


Fig 1 Density of submucous glands in the nasal septum in nasal allergy and in normal adults

rhinitis than in normals, but quantitation was not possible on the basis of the small biopsies

Owing to the polymorphous appearance it would be difficult to make further progress in the study of the gland pathology by conventional histological methods We therefore started quantitative studies on the glands, using whole-mount methods on a normal adult autopsy series (Tos & Mogensen 1976*a*, *b*) Thereby, we found a patient with allergic rhinitis who afforded us a possibility of elucidating the nasal glands in this disease from new aspects

MATERIAL AND METHODS

The patient, who died at the age of 18 of metastasizing cancer of the testis, had a history of typical attacks of hay fever lasting from May until August In between the periods of hay fever, he had had increased mucous nasal secretion and once an episode of sinusitis

Post mortem examination revealed mucopurulent secretion in both nasal cavities, diffuse thickening of the mucosa, deviation of the septum to the left, and a hypertrophic middle concha on the right He had never had nasal polyps

The entire mucosa from the septum and conchae was removed fine-dissected, and stained by the PAS–alcian blue whole mount method (Tos & Mogensen 1976*a*) The septum was divided anteroposteriorly into four quarters and inferosuperiorly into three parts, making a total of 12 localities Each concha was divided anteroposteriorly into three parts and into a superior and inferior half, making 6 localities in the medial and lateral wall In each locality 3–6 counts of glandular orifices of the subepithelial glands were made in the stereomicroscope, magnification ×50, in fields measuring 4 mm² Thereafter, the median density (glands/mm²) in each locality was calculated

From several parts of the nose the mucosa was cut into serial sections and stained with haematoxylin eosin PAS–alcian blue and by combinations of these methods

RESULTS

Density of Glands

Nasal septum In all quarters the median density of the orifices of seromucous glands was significantly higher than in a normal septum (Tos & Mogensen, 1976*a*) (Fig 1) In the anterior half of the septum the density was greater than in the posterior half In the inferior third the density was lowest (Fig 2), in the upper part of the third quarter it was particularly high

Inferior and middle conchae In both, the median density was far higher than in 13 normal conchae (Fig 3) (Tos & Mogensen 1976*b*) This applies to the medial (septal) as well as to the lateral walls There was a very wide range in density In the middle

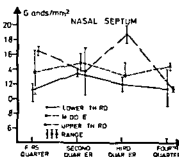


Fig 2 Density of glands in the lower middle and upper thirds of the septum in nasal allergy

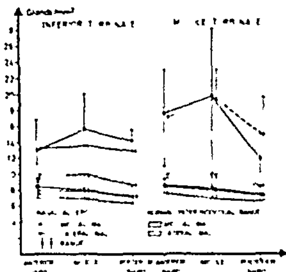


Fig. 3. Density of glands in the conchae in nasal allergy and in normal adults.

concha the density was greater in the inferior than in the superior half (Fig. 4), while no major differences were found in density in the inferior concha.

The explanation for this high density must be that new glands have formed in the course of the patient's long-lasting nasal disease.

Distribution of orifices

On the whole mounts it was seen that the distribution of the orifices of subepithelial glands was—unlike that in a normal nose—very irregular, being grouped in certain areas

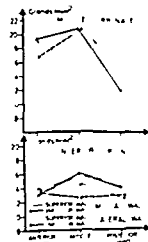


Fig. 4. Density of glands in the superior and inferior halves of the conchae in nasal allergy.



Fig. 5. (a) Mucosa of the inferior concha. The orifices are of irregular distribution. High density anteriorly. PAS-alcian blue whole mount. ×50. (b) Many large orifices in an area with high density. (c) Fewer large orifices in an area with lower density. ×40.

(Fig. 5a). Thus, the formation of new glands cannot have been equally intense throughout the mucosa.

Most orifices were larger than in a normal nose, measuring 100–150 μm in diameter. The orifices and the main ducts, which were lined with mucous cells, were rather clearly visible as round, blue rings in a surface epithelium with few goblet cells (Fig. 5b, c). Several orifices and ducts—a considerably larger number than in normals—were filled with intensely stained mucous secretion, bearing witness to intense glandular activity. The secretion often appeared as a round patch on the epithelium around the orifice, indicating lack of ciliary function. Rarely, there was a streak of mucus from the orifice and posteriorwards.

Beside the orifices there were in a few sites on the septum many small, irregularly distributed intra-epithelial glands. Such glands are observed here and there in normal adult noses but not in foetuses, prematures, or neonates.



Fig 6 (*a*) Normal superficial and (*b*) normal deep-lying gland The epithelium is very thick stratified with papilliform prominences (*arrows*) H–E ×400

Gland structure

On serial sections two types of subepithelial glands could be distinguished (*a*) Glands with narrow main and side ducts, made up of simple, cuboidal, secretory, predominantly inactive epithelium (Fig 6) The side ducts divided into tubules ending in acini, both lined with simple epithelium consisting of predominantly serous or predominantly mucous cells In structure these glands resembled normal sero-mucous glands (*b*) Glands whose main duct was made up of thick pseudostratified epithelium containing in places many, large, distended mucous cells (Fig 7*a*) The calibre of the main duct was wide, uneven having localized distensions (Fig 7*b* *d*) In the proximal segments of the side ducts the epithelium was also pseudostratified, while distally it gradually grew thinner and was two-layered (Fig 7*c*) The tubular epithelium was simple, one layered, consisting predominantly of columnar mucous cells The tubules ended in serous, mucous, or mixed acini These glands, hereafter called "abnormal", occurred side by side with normal glands in all parts of the nose In numbers per section their density agreed with that found in the whole mounts

On the whole, both types of glands exhibited signs of hyperactivity of the acini and tubules and quantitative predominance of serous over mucous acini In places there were, deep down in the lamina propria, round structures reminiscent of acini, but without a lumen and consisting of undifferentiated cells, which possibly indicates new formation of glandular tissue Rarely, there was major distention of the acini or tubules with stagnation of secretion and atrophy of the secretory cells into simple, flat epithelium

As for other histo-pathological changes marked hyperplasia of epithelial cells and subsidence of ciliated cells were the most outstanding (Figs 6, 7) In all segments the pseudo-stratified epithelium was greatly thickened, having several layers of round basal cells and columnar cells superficially In many sites there were localized thickenings of the epithelium, projecting down into the lamina propria (Fig 6*a*) Cilia were hardly ever seen in the anterior half, rarely in the posterior half In the lamina propria there was pronounced infiltration of eosinophilic cells and cells with round nuclei and migration of these cells through the moderately thickened basement membrane and epithelium There was marked thickening of the walls of large as well as small vessels

DISCUSSION

It has been demonstrated by Swindle (1935) that water injected into vessels in normal,



Fig. 7. Abnormal glands. (a) stratified duct epithelium with large mucus cells. (b) distention of the duct with a debouching tubule (arrow). (c) pseudostratified duct epithelium (arrow). (d) distention of the main duct lined with stratified epithelium with a debouching tubule (arrow). H.E. ×400.

dead reindeer transuded through the vessels and epithelium to the surface. Ingelstedt & Ivstam (1949*a*), using the fluorescein method, were unable to demonstrate transudation of fluorescein to the surface. They felt that the nasal secretion in a normal human nose was derived exclusively from the nasal glands. In patients with acute nasal infection and in patients with hay fever they found in the same experiments (Ingelstedt & Ivstam 1949*b*) fluorescein in the nasal secretion, indicating that in these conditions the secretion is—at least partly—an exudate or a transudate. In chronic nasal allergy, on the other hand, they found no fluorescein in the secretion, which was said to be exclusively a product of glands. In fluorescein experiments on animals in histamine shock, Messerklinger (1958) found no proof of transudation, but fluorescein in the glandular tissue. He deduced that under normal as well as abnormal conditions the secretion is produced by glands. He rejected previous theories of transudation—through the colloidal membranes of the epithelium (Sternberg 1927) and due to high tissue pressure in the mucosa (Negus 1934)—stating that the nasal secretion is not an ultrafiltrate having the same concentration of proteins as the serum. In more recent electron microscopic studies of pollen sensitized rabbits during acute anaphylactic reaction, Terrahe & Backwinkel (1970) demonstrated the passage of tracer protein (peroxidase) from capillaries through the basement membrane and epithelium to the mucosal surface. In non-sensitized controls the tracer protein was arrested at the epithelial barrier. In these authors' opinion transudation is an important source of secretion in acute allergic states, though not under normal conditions. In patients with chronic nasal allergy and during attacks of hay fever, Jahnke (1972) and Lenz (1972) found the epithelium to contain distended intercellular spaces filled with transudate.

Our findings show that owing to the formation of new glands the number and density of glands are greatly increased in nasal allergy. As the duct system of these glands is lined with highly secretory epithelium, and as the number of tubules and acini belonging to these glands is greater than that of most normally occurring glands, they represent an appreciable augmentation of secretory capacity by the nose. As the original glands also exhibit hyperplasia and hyperactivity of the acini—a phenomenon also observed by others (Hansel 1930, Terrahe 1970, Jahnke 1972)—there is a patho-anatomical basis for assuming that the greatly increased secretion in nasal allergy is derived exclusively from existing glands. However, these findings do not exclude the

possibility of transudation during an acute attack of hay fever Owing to the greatly thickened epithelium, however, the facilities for transudation must be much poorer than in a normal nose with thinner epithelium

In the presence of a sudden increase in aqueous secretion during attacks of hay fever, the secretory capacity of the serous Bowman glands from the olfactory region must be taken into consideration Very little is known about these glands, apart from the fact that they are small and tubular, but in foetuses they are of greater density than are the sero mucous glands in the respiratory region (Tos & Mogensen, 1976c) It is conceivable that the stimulus which suddenly activates the glands in the respiratory region and the lacrimal glands may also activate the glands of Bowman

The serous anterior nasal glands (Bojsen Møller, 1965) are few and fairly small As compared with the other glands, their share in secretory production is minimal, and they represent mainly a phylogenetic rudiment (Tos & Poulsen, 1974) In our case there was no special accumulation of serous acini that could be ascribed to these glands anteriorly in the nose

Goblet cells, which according to several authors (Jahnke, 1972, Mygind & Bretlau 1974, Mygind et al , 1974) are increased in an allergic nose, were not increased in our case If anything, they were decreased in relation to a normal nose, but an exact quantitative determination of their density is needed

Probably most of the glands with stratified duct epithelium, which we call abnormal were formed in the course of the disease They were of an extremely irregular distribution and were observed to be, in whole mounts as well as in sections, concentrated in the very sites where the density is greatest The normal glands on the other hand were uniformly distributed over the entire mucosa On the other hand, it may be imagined that epithelial metaplasia into stratified squamous epithelium continues down into the ducts In the vestibule, for instance, most ducts were lined with stratified epithelium In other areas normal glands were seen side by side with abnormal ones

How and why do the new glands form? In the foetal nose the undifferentiated epithelial cells grow down into the depth, forming a solid cylinder which undergoes dichotomous division and becomes canalized as the cells differentiate (Tos & Poulsen, 1974) In secretory otitis, abnormal glands form in the middle ear in the process of hyperplasia and metaplasia of simple into stratified epithelium, in the same way (Tos & Bak Pedersen, 1975), and the tubules become lined with stratified columnar epithelium As in the present case there was also considerable hyperplasia of the basal epithelial cells with papilliform prominences down towards the lamina propria, it is conceivable that new formation of glands in the nose too is a link in hyperplasia of the epithelium whose cells penetrate the basement membrane grow down into the propria, whereupon they differentiate and the gland becomes canalized The lining of the abnormal glands, and the ducts with pseudostratified epithelium could thus be explained, but probably glands can form also during other non allergic, hyperplastic diseases of the nose

ZUSAMMENFASSUNG

Die ganze Nasenschleimhaut von einem Patienten mit Nasenallergie und Heufieber wurde nach der Ganzpraparatmethode untersucht und die Dichte der submukosen Drusen bestimmt Die Dichte was in allen Abschnitten der Nase besonders in den Muscheln wesentlich höher als in der normalen Nase welches darauf hindeutet daß wahrend der Krankheit neue—pathologische—Drusen gebildet werden die sich auch morphologisch von den normalen Drusen unterscheiden Die sekretorische Kapazitat der Schleimhaut ist dadurch stark gesteigert und die pathologisch anatomische Grundlage fur die Auffassung daß das ganze Sekret bei der Allergie von den Drüsen gebildet wird ist gegeben Andere Quellen zur Nasensekretion im normalen und pathologischen Zustande die Exsudation und Transsudation sowie die Ursache und Weise der Drusenneubildung wurden diskutiert

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IgA IMMUNOCYTES IN TONSILS

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Abstract Dimeric IgA forming cells were studied by a secretory component (SC) affinity test on 20 palatine and 7 pharyngeal tonsils from children This study also included an investigation on the immunofluorescent localization of IgA immunocytes and IgA and SC deposits The results showed that IgA immunocytes capable of binding SC in tissue sections are present in both palatine and pharyngeal tonsils However the number of cells positive for the SC affinity test was significantly lower than that of IgA immunocytes not binding SC IgA immunocytes were located mainly in the subepithelial area medullary portion and occasionally the intraepithelial layer SC determinants were detected only in some epithelial cells of the pharyngeal tonsils The findings of the present study suggest that the pharyngeal tonsils share in the local immunological mucosal resistance regulated by secretory IgA although its activity might be limited

IgA is the predominant immunoglobulin in external secretions and plays an important role in immunological mucosal resistance It is known that there are two types of IgA serum IgA and secretory IgA The latter possesses an epithelial glycoprotein called the secretory component (SC) In the past it has been suggested that secretory IgA is synthesized in such a way that the locally produced IgA which is probably identical with 7S serum IgA traverses the mucosal membrane or glandular epithelium where SC is produced and forms a complex with SC (Tomasi & Bienenstock 1968) However since evidence from recent studies (Mach 1970 Brandtzaeg 1971) showed that free SC (unassociated with IgA) combines *in vitro* with serum IgA (10S IgA having J chain but not monomer type) to produce 11S secretory IgA, and since J (joining) chain and IgA were found in the same plasma cells of lamina propria (O Daly & Cebra 1971 Parkhouse, 1972), it is suggested that 10S IgA molecules formed by plasma cells in the submucosa are transported to the epithelial cell layer and secreted to the luminal secretions

Crabbe & Heremans (1967), Tada & Ishizaka (1970) Ishikawa et al (1972) and Mogi (1975) have all found that the tonsils have cells producing immunoglobulins of all five classes Mogi (1975) observed that whereas SC or secretory IgA do not exist in the palatine or lingual tonsils some columnar epithelial cells of the pharyngeal tonsil contain SC determinants The epithelium of the pharyngeal tonsil is of the respiratory type This evidence therefore suggests the presence of local immunological mucosal resistance in the pharyngeal tonsil However, it is not known whether IgA forming cells accumulating in the basement membrane of the palatine tonsil produce 7S or 10S IgA

The present study was designed to identify two types of IgA immunocytes which could be distinguished by a SC affinity test Immunofluorescent localization of IgA forming cells and IgA and SC deposits are also investigated

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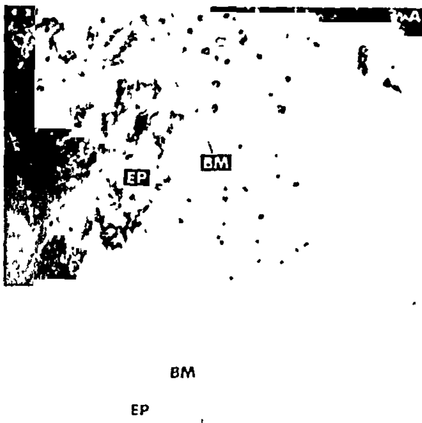


Fig. 1. The immunohistochemical SC affinity test performed on IgA immunocytes in a pharyngeal tonsil section. (A) A tissue section treated with anti-IgA conjugate (×125). IgA-forming cells are scattered throughout the subepithelial layer. Bright cytoplasmic staining of epithelial cells can be seen. (B) An adjacent section of the same tissue specimen incubated first with SC and thereafter with anti-SC conjugate (×125). There are several immunocytes binding to SC in the subepithelial portion (*arrow*). Epithelial cells react with anti-SC conjugate. *EP*, epithelium; *BM*, basement membrane.

MATERIAL AND METHODS

Twenty palatine and 7 pharyngeal tonsils were obtained by surgery from children (ranging from 4 to 15 years of age) who underwent tonsillectomies and/or adenoidectomies because of recurrent tonsillitis or hypertrophy of the tonsils.

Isolation of secretory IgA, free SC and lactoferrin

The fractionation of secretory IgA and free SC from human colostrum was carried out as described previously (Mogi, 1975). Lactoferrin was purified from human colostrum using CM-Sephadex (Pharmacia Fine Chemicals, Uppsala, Sweden) chromatography by the method described by Kobayashi (1974). Each product was submitted to immunodiffusion and immunoelectrophoresis and was confirmed to have no contaminating proteins.

Rabbit IgG

Rabbit IgG was purified from the serum of a normal rabbit by DEAE-cellulose chromatography with a 0.01 M phosphate buffer at pH 7.4.

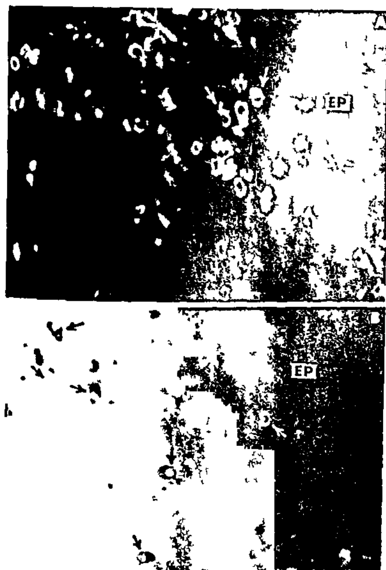


Fig 2 The immunohistochemical SC affinity test performed on IgA immunocytes in a palatine tonsil section (A) A tissue section treated with anti IgA conjugate (×312) The arrow indicates an IgA immunocyte (B) An adjacent section of the same tissue specimen incubated first with SC and thereafter with anti SC conjugate (×312) Note that the number of IgA immunocytes positive for the SC affinity test *(arrow)* is significantly smaller than that of the IgA immunocytes *EP* epithelium

Antisera

Antisera to human secretory IgA and SC were prepared in rabbits as reported in a previous paper (Mogi, 1975) Antiserum to lactoferrin was prepared by immunizing rabbits with the lactoferrin preparation in complete Freunds adjuvant at 7 day intervals over a period of one month

Fluoresceinated antisera

Conjugation of each antibody to secretory IgA, SC and lactoferrin with fluorescein isothiocyanate (FITC) was done as described previously (Mogi 1975) FITC-conjugated rabbit antibodies to human IgA (α-chain) and IgG (γ-chain) were purchased from Behringwerke Marburg, Lahn, Germany The

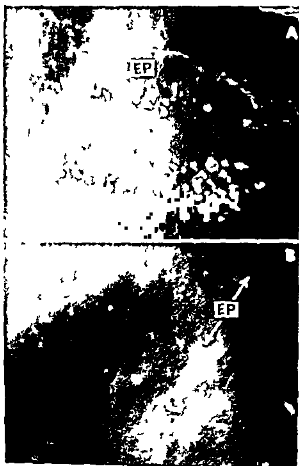


Fig 3 The immunohistochemical SC affinity test performed on IgA immunocytes aggregating in the subepithelial area of a pharyngeal tonsil (A) A tissue section treated with anti IgA conjugate (×125) IgA immunocytes are aggregating (B) An adjacent section of the same tissue specimen incubated first with SC and thereafter with anti SC conjugate (×312) Only one cell of the aggregating IgA immunocytes is *positive for the SC affinity* test *EP* epithelium

bodies were allowed to absorb human liver powder to prevent non specific staining for connective tissue

SC affinity test and immunofluorescence studies

Specimens of the fresh tissues were fixed in cold ethanol (95%) for paraffin sectioning as reported previously (Mogi 1975) Consecutive sections approximately 4 μm thick were obtained from each tissue The SC affinity test was done by the method reported by Brandtzaeg (1973) The purified free SC was dissolved at 20 μg/ml in a 0 3 (w/v) solution of rabbit IgG in PBS (phosphate buffer saline at pH 7 2) Serial tissue sections were incubated with this reagent for one hour at 37°C and then overnight at 4°C After a 30 minute wash with 3 changes of the PBS, the sections were incubated immediately with FITC labelled antibodies to SC for one hour in a 37°C moist chamber The sections were rinsed in PBS washed for one hour with three changes of PBS, and mounted with non fluorescein glycerin Control sections were incubated with a 0 3% solution of rabbit IgG alone, and with a lactoferrin solution with a concentration of 20 μg/ml in a 0 3% solution of rabbit IgG in PBS Sections incubated with the lactoferrin solution were treated with FITC-labelled rabbit antibodies to lactoferrin Sections incubated with rabbit IgG solution alone were treated with FITC-labelled antibodies to SC and FITC-labelled antibodies to lactoferrin respectively

Staining with each FITC labelled antibody to secretory IgA, α-chain, SC, and γ-chain was done as previously described (Mogi 1975) Blocking tests were also performed by applying the appropriate unconjugated antibodies to the consecutive tissue sections prior to staining with FITC-labelled antibodies The specimens were examined with a Leitz incident light fluorescence microscope (Dialux Leitz, Wetzlar, Germany) using KP500 and BG38 excitation filters and a dichroic beam splitting mirror (TK 510) and suppression filter (K515) Paraffin sections from each block were obtained with HE for light microscopy

RESULTS

The cytoplasmic SC affinity of IgA immunocytes

Although there were apparently fewer positive cells than IgA immunocytes not binding SC, positive immunocytes for the SC affinity test were detected in sections of both palatine and pharyngeal tonsils The intensity of fluorescent staining in the SC affinity test varied be-

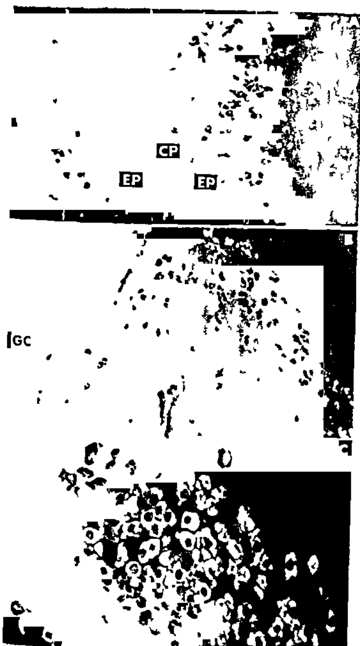


Fig 4 Distribution patterns of IgA immunocytes in tonsils (A) IgA immunocytes accumulate in the subepithelial area and some of them are located in the epithelial layer *(arrow)* (×122) (B) Fewer IgA immunocytes are seen further from the tonsillar epithelium No IgA immunocytes are detected in the germinal center (×122) (C) IgA immunocytes from an aggregation in the subepithelial portion (×305) *EP* epithelium *CP* crypt *GC* germinal center

tween positive cells in the same section as well as between different samples Generally speaking the positive cells were more common in the pharyngeal tonsils than in the palatine Fig 1 shows adjacent sections of a pharyngeal tonsil treated with anti IgA conjugate (A) and the results of the SC affinity test (B) In this area the number of positive immunocytes for the SC affinity test was nearly the same as that of IgA forming cells Both the SC affinity positive immunocytes and IgA forming cells were scattered in the subepithelial region and cytoplasmic staining of epithelial cells was clearly seen in both sections Fig 2 presents adjacent sections of a tonsil treated with anti IgA conj

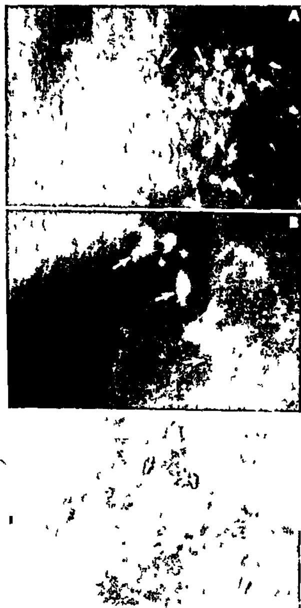


Fig 5 Immunofluorescent localization of IgA and SC determinants in the epithelium of a pharyngeal tonsil The arrow indicates cytoplasmic fluorescence (A) A tissue section treated with anti IgA conjugate (×280) (B) A tissue section treated with anti SC conjugate (×280) Note the bright granular fluorescence in the cytoplasm of cells relatively close to the basement membrane (C) A tissue section treated with anti SC conjugate (×280) showing brilliant fluorescence in the apical cells *EP* epithelium *CP* crypt *GC* germinal center

the SC affinity fluorescence (B) IgA forming cells were abundantly present but not aggregated In this area, positive immunocytes were relatively rare However as shown in Fig 3, the positive immunocytes for the SC affinity test were extremely rare in areas of aggregated IgA forming cells A single immunocyte positive for the SC affinity test was rarely observed, regardless of the existence of IgA-forming cells in the specimen, in either palatine or pharyngeal tonsils Weber's glands were observed in the tissue sections of two out of 20 palatine tonsils which were submitted to the SC affinity test However, positive fluorescence was not detected in either the acinal cells or lumens of the glands Treatment with anti lactoferrin conjugate did not produce any bright fluorescent stain The control sections incubated with rabbit IgG alone and then stained with SC conjugate showed no fluorescence in any area of the palatine tonsils examined, whereas clear cytoplasmic staining of epithelial cells was seen in sections of the pharyngeal tonsils However, in the control sections of the pharyngeal tonsils stained with anti SC conjugate, no fluorescence was seen in the basement membrane, subepithelial area medullary portion, germinal center, or fibrous septum Other control sections incubated with lactoferrin and then treated with anti lactoferrin conjugate produced no fluorescence in any area of the palatine or pharyngeal tonsils

Immunofluorescent localization of IgA-forming cells and IgA and SC deposits

The distribution pattern of IgA-forming cells was consistent in specimens of both palatine and pharyngeal tonsils The most abundant immunocytes observed in tonsils were IgG forming cells followed in predominance by IgA forming cells The number of IgA immunocytes varied between different specimens As shown in Fig 4, IgA immunocytes were distributed mainly in the subepithelial area medullary portion and occasionally in the intra epithelial layer IgG immunocytes were also located in these areas Even though germinal centers were found to contain IgG immunocytes on occasion, there were few IgA



Fig 6 A tissue section of a pharyngeal tonsil treated with anti secretory IgA conjugate (×125) Note the heavy accumulation of IgA immunocytes *(single arrow)* approximating the basement membrane and the bright cytoplasmic staining in the epithelial cells *(double arrow)* *EP* epithelium *GC* germinal center

immunocytes in the germinal centers In general fewer IgA immunocytes are seen further from the tonsillar epithelium and the membrane lining crypt (Fig 4*B*) As can be seen in Fig 4*C*, IgA immunocytes frequently form an aggregation in the subepithelial portion close to the basement membrane In pharyngeal tonsils cytoplasmic staining of epithelial cells with anti IgA conjugate and anti-SC conjugate is seen (Fig 5) Bright, granular fluorescent staining for SC is apparent in the cytoplasm of epithelial cells relatively close to the basement membrane (Fig 5*B*) Fig 5*C* shows brilliant fluorescence in apical cells of the epithelial layer Fig 6 is an immunofluorescent photograph of a pharyngeal tonsil treated with anti secretory IgA conjugate showing a heavy accumulation of IgA immunocytes approximating the basement membrane of the epithelial layer in which bright cytoplasmic stains are seen However in the sections of some pharyngeal tonsils treatment with anti secretory IgA conjugate revealed that cytoplasmic or intercellular reaction was very faint in the epithelial layer in spite of the heavy accumulation of IgA immunocytes in the subepithelial portion The reverse phenomenon, bright fluorescent staining of the epithelial cells without presence of IgA immunocytes in the submucosal area, also occurred In the epithelial layer of palatine tonsils positive staining with anti IgA or anti IgG conjugate was usually absent or very faint However, heavy diffused staining for IgA and IgG was seen occasionally in the cytoplasm and intercellular space of some squamous epithelia in palatine tonsils The heavy epithelial staining occurred regardless of accumulations of corresponding immunocytes Cryptal surfaces and masses were occasionally positive for IgA and IgG but not for SC in the palatine tonsils

DISCUSSION

Since Tomasi et al (1965) identified secretory IgA there have been a number of studies suggesting that this class of antibody contributes significantly to the immunological mucosal resistance mechanism Secretory IgA is synthesized locally in the gastrointestinal tract, salivary glands mammary glands,
tract (including the middle ear) and urc

organs However, conflicting data have appeared concerning the existence of secretory IgA in tonsils Rossen et al (1968) reported the presence of secretory IgA in the membranes lining the crypts and subepithelial cells of palatine and pharyngeal tonsils, whereas no SC determinants were found in palatine tonsils by the studies of Schmedtje & Batts (1973) Mogi (1975) reported that SC is synthesized in the epithelial cells of pharyngeal tonsils but not in any portion of palatine tonsils This was confirmed by the present study

As Mach (1970) and Brandtzaeg (1971) showed that free SC combines *in vitro* with *10S serum IgA having J chain (but not 7S* monomer IgA) producing 11S secretory IgA, it has been suggested that there are two types of IgA immunocytes, monomer IgA-forming cells and dimer IgA-forming cells Brandtzaeg (1973) first demonstrated the presence of these two types of IgA immunocytes by the SC affinity test According to his study, in the salivary glands and colon, where secretory IgA is abundantly produced, there was a preponderance of 10S IgA forming cells Although four palatine tonsils were included in his study, Brandtzaeg (1973) found that the presence in palatine tonsils of 10S forming cells was rare and that most IgA immunocytes adjacent to crypt or the surface epithelium did not bind free SC Our present study showed that the number of IgA immunocytes capable of binding SC in palatine tonsils was smaller than that in pharyngeal tonsils However it was evident that palatine tonsils contain dimeric IgA-forming cells although not many IgA immunocytes usually exist in the subepithelial or medullary portion of the tonsils frequently forming aggregations of cells apparently clones Most of the aggregating IgA forming cells did not bind SC However it is still not clear whether or not these two cell types belong to different cell lines or different developmental stages of the same line Brandtzaeg (1973) considered that immunocytes producing dimeric IgA seem to be characteristic of tissue containing SC producing epithelia

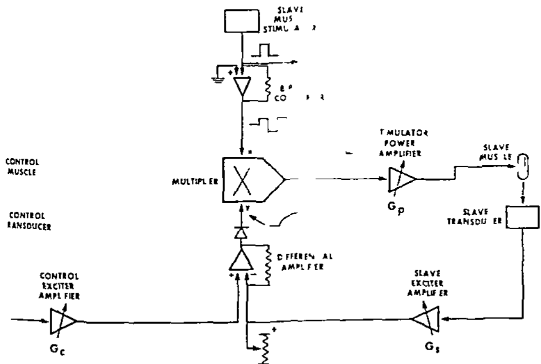
Mestecky et al (1972), Kobayashi et al (1974) and Eskeland & Brandtzaeg (1974) have identified J chains in molecules of polymeric IgA and IgM Brandtzaeg (1973) showed that free SC also combines readily with 19S IgM. Therefore, there is a possibility that some immunocytes positive for the SC affinity test in the present study might be IgM immunocytes However, Mogi (1975) reported that a few IgM-forming cells are present in every 10 fields (at 312 magnification) of tonsil sections and that IgM-forming cells are apparently not localized in any particular site

It was observed in this study that in pharyngeal tonsils SC existed in the cytoplasm of both epithelial cells close to the basement membrane and of apical cells This finding suggests that pharyngeal tonsils secrete IgA in such a way that dimeric IgA produced in the subepithelial portion is bound with free SC in the epithelium and secreted Eskeland & Brandtzaeg (1974) strongly suggested that SC has specific affinity to the J chain of polymeric immunoglobulins However, this secretory activity of IgA in pharyngeal tonsils is not remarkable, since it was apparent in this study that positive epithelial cells for SC and the number of IgA immunocytes producing dimeric IgA are limited

Lactoferrin is an antibacterial iron binding protein in milk and other external secretions The present study failed to detect lactoferrin in either palatine or pharyngeal tonsils Masson et al (1966) demonstrated, in their immunohistochemical study on lactoferrin in bronchial mucus, that this protein is present in glandular acini whereas neither the epithelial lining nor the alveolar wall appears to take part in its production

ZUSAMMENFASSUNG

Die IgA Dimere produzierenden Zellen wurden mittels Affinitatstests gegen die sekretorische Komponente (SC) auf 20 Stuck Gaumen und 7 Stuck Rachentonsillen untersucht die durch Tonsillektomie aus Kindern genommen wurden Lokalisierung von Immunofluoreszenz von IgA Immunozyten und Ablagerung von IgA und SC wurde auch mit denselben Tonsillen untersucht Die IgA Immu



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METHODS

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CONTROL OF PAR[illegible]SED AXIAL MUSCLES BY ELECTRICAL STIMULATION

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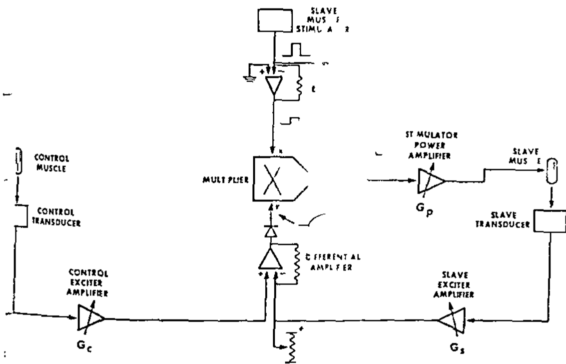
From the Depar[illegible] [illegible]ngology and Physiology, University of California at San Francisc[illegible] [illegible]al Laboratory–HSE 853, San Francisco, Ca. USA

(Received June 3, 1976)

Abstract. The function of a paraly[illegible] facial, extraocular) muscle could [illegible] if it were made to contract ag[illegible] partner. A muscle stimulation devi[illegible] constructed so that the stimulus d[illegible] paralysed muscle was modulated by a [illegible] contractile state of its contralateral partner [illegible] laryngeal muscles indicate that paralysed [illegible] lated by such an open loop device co[illegible] partners. However, their tracking accuracies [illegible] by the nature of their stimulus-response c[illegible] On the other hand, significantly greater tr[illegible] racies were observed if a closed-loop devi[illegible] ployed, that is, if feedback information from [illegible] lated muscles was also used to control the dev[illegible] ulus level. Considerations in implanting such a [illegible] loop or open loop) device in paralysed axial mus[illegible] chronic stimulation are discussed.

Palsies of the axial muscles of the head and neck result from lesions of the central nervous system, or of peripheral nerves innervating the muscles. At the present time, there is no satisfactory treatment of muscle paralysis arising from a CNS lesion. In the case of a severed peripheral nerve, regeneration to its paralysed muscles is encouraged, where possible, by anastomosis of the two nerve stumps. Unfortunately, in many cases an anastamosis with the intrinsic nerve or with a foreign nerve cannot be performed. Even when an anastamosis is made there is uncertainty that motoneurons will reinnervate their original muscles. In a crush injury to a nerve, budding neur[illegible] their Schwann sheaths in the distal de[illegible] ated segment to guide growth back t[illegible] muscle. Thus, when a transected pr[illegible] stump is apposed to the distal stump, [illegible] neurons come to occupy other sheaths [illegible] often lead to other muscles (Ochs, 1965[illegible] degree to which these neurons are segr[illegible] from other neurons destined for other m[illegible] and how nerve stumps are approximate[illegible] important factors in determining the ext[illegible] crossed reinnervation. Statistically, app[illegible] mately 50% of patients have "good" retu[illegible] function after a facial nerve anastamosis [illegible] all control is fair and movements of the [illegible] are of mass quality in these patients (F[illegible] 1974). Results are less successful in the cas[illegible] a recurrent laryngeal nerve anastamosis ([illegible] dies have been primarily limited to exp[illegible] mental animals). Reinnervation occurs but [illegible] turn of normal vocal fold mobility is rar[illegible] observed (Dedo, 1970, Dedo, 1971, Muraki [illegible] & Kirchner, 1971; Tashiro, 1972). Anatom[illegible] studies indicate that motoneurons are [illegible] segregated within the recurrent laryn[illegible] nerve until it enters the larynx (Sunderland [illegible] Swaney, 1952). Thus, crossed reinnervati[illegible] presumably accounts for these results. Norm[illegible] vocal cord motion is evidently dependent upo[illegible] the balance in tensions in laryngeal muscle an[illegible] tagonists; crossed reinnervation frustrates re[illegible] establishment of this balance.

This work was supported by the National Institute of Health Grant no. NS06527.



1 Block d agram of the muscle stimulation device ..ployed in these studies

How might one restore function to individ paralysed muscles? Ideally, a paralysed iscle would be reinnervated specifically and ectively with its own motoneurons How r this is a formidable task, especially in w of the uncertainty in knowledge of fac s affecting regeneration A possible alterna e approach is based on the fact that axial iscles are in general bilateral and function as rs It is possible that paralysed laryngeal ial or extraocular muscles could be made contract by direct electrical stimulation con lled by the state of contraction of homol ous contralateral muscles Such an ap oach might be useful in cases of peripher il rve lesions where nonspecific reinnervation the intrinsic or a foreign nerve has oc rred Furthermore it might be useful in atment of unilateral CNS lesions where no ier treatment is available A muscle stimula n device has been constructed and tested in yngeal muscles in dogs These studies indi e that a paralysed axial muscle can be brought under the control of its normal contra lateral homologue Considerations in implant ing of such a device for chronic use will be discussed

METHODS

Muscle stimulation device

A block diagram of the muscle stimulation de vice used in these studies is shown in Fig 1 Given paralysed muscles were stimulated by a biphasic square wave train the magnitude of which was modulated (by the multiplier) as a function of the tension of contralateral homol ogous muscles That is, a paralysed muscle became a "slave' to its contralateral (control ling) muscle The square wave train was gen erated by the slave muscle stimulator and made biphasic and symmetrical by the bi ph isic converter One could be assured that the stimulus voltage the output of such a lin ear open loop device would vary in tion to the tension of the control 2) However, there was no

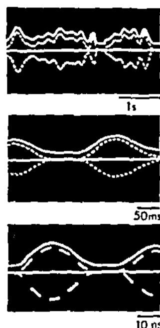
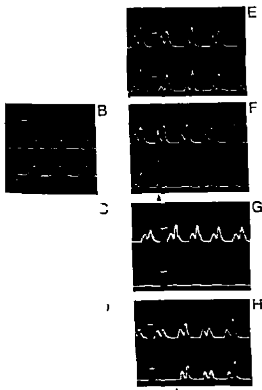


Fig 2 Comparison of stimulus voltage and control muscle tension at three different sweep speeds Biphasic stimulus=broken traces control muscle tension solid traces

slave muscle tension would vary so as to accurately match control muscle tensions unless there was also a linear relationship between stimulus voltage and slave muscle reponse magnitude It was anticipated that compensation for any nonlinearity in the slave muscle stimulus–response characteristics and better tracking of control muscle tensions by the slave muscle might be achieved if the tension of the slave muscle was also monitored and the difference or error in tensions between the two muscles used to modulate the stimulus strength as diagrammed in Fig 1 The muscle stimulation device was constructed with the flexibility to be operated in either closed loop or open loop mode that is with or without feedback information from the stimulated slave muscle In particular if G_s (the slave exciter amplifier gain) was varied relative to G_c (the control exciter amplifier gain) the amount of feedback could be modified Thus one could obtain the open loop mode from the closed loop mode by reducing G_s to zero Alternatively the feedback loop could be disconnected from the differential amplifier to obtain the open loop mode G_p (the stimula power amplifier gain) was also made varia in the construction of this device so that open loop or closed loop device gain could modified when desired in order to obtain reference slave muscle response for a gi control muscle response The tension tra ducers used in these studies (Grass for displacement transducers FTO3C) were cited and their bridges balanced by the citer amplifiers

Animal preparation

Acute studies were performed on the cri thyroid muscles of 11 adult dogs Anim were initially anesthetized with sodium pen barbital and an intravenous catheter intı duced for further administration of anesthe during the course of the study A midline cision was made in the neck exposing t strap muscles which were dissected den vated and cut to expose the cricothyroid mu cles on each side The recurrent laryngeal a superior laryngeal nerves were identified a dissected free from fascia loose ligatures we placed around each nerve for later retriev Each medial cricothyroid muscle was then d sected free from underlying ligamentous a tachments by a medial approach Since is metric tensions of the muscles were mo tored in this study the thyroid cartilage w anchored to a stationary stainless steel rod v screws in the thyroid laminae while the i ferior end of each medial cricothyroid musc was attached to a calibrated tension tran ducer via a part of the cricoid cartilage Gre care was taken to prevent disruption of tl innervation and blood supply to each musc during dissection To reduce movements the larynx (and thus tension artifacts) duri respiration the trachea was transected and stiff tracheal cannula with a sidearm inserte The tracheal cannula allowed air flow throu the larynx or alternatively through the e ternal sidearm The trachea was then imm bilized by fastening the cannula via a stai.. less steel rod to the animal table



3 Restoring "natural function to a paralysed cricoroid muscle with the muscle stimulation device In all upper traces are the tensions developed by the cricothyroid muscle In all frames except B through lower traces are tensions developed by the left cricoroid muscle In frames B through D lower traces de ibe the respiratory cycle which was monitored with a tysmographic transducer around the chest Calibration nals=100 grams tension 1 second duration

The transducers were positioned to rein resting length in each muscle (where sting length is defined as the length at which tive tension produced becomes maximum hen stimulus strength is held constant) The idges were balanced and a supramaximal imulus delivered to each muscle The muscle iat exhibited greater tension was used as the ave muscle in the study to assure that it 'd match any tension attained by the con rol muscle when contracting maximally Ob ions were then made of the tensions during natural contractions of both scles during respiration After these pre iminary observations of normal muscle ac tion the external branch of the supe ryngeal nerve innervating the slave muscle was cut to induce paralysis A pair of barbed stainless steel electrodes (leading to the muscle stimulation device) was placed across the belly of the slave muscle, and the device employed to determine whether or not the function of this paralysed muscle could be restored

After these studies were completed, the performance of the slave muscle was observed in detail (utilizing each device mode) as a variety of complex responses were induced in the control muscle by electrical stimulation These complex responses were evoked by stimulation with a pair of barbed stainless steel electrodes placed across the belly of the con trol muscle and driven by a Grass stimulator To eliminate natural laryngeal muscle contrac tions the superior laryngeal nerve innervating the control muscle and both recurrent laryn geal nerves were cut The sidearm of the tracheal cannula was then opened to allow effortless breathing

RESULTS

I *Tracking of Natural Contractions of Control Muscle by Paralysed Slave Muscle*

Dogs were maintained at a light plane of anes thesia during this phase of the study so that they would contract their cricothyroid muscles during respiration Both right (upper trace) and left (lower trace) cricothyroid muscles can be observed contracting in the study shown in Fig 3 A When the respiratory cycle was mon itored as shown in the lower trace of Fig 3 B the contractions of each muscle were found to occur during inspiration (the first ar row marks the onset of inspiration the second arrow the onset of expiration) However at lighter planes of anesthesia the muscles also contracted during expiration so that each muscle s activity became biphasic with the re spiratory cycle (Fig 3 C) The inspiratory con tractions were small (less than 100 grams ten sion) with little variation in magnitude in any given animal These contractions probably

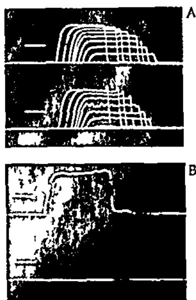


Fig 4 (A) Tracking of control muscle fused tetanus plateaus (upper traces) by the slave muscle (lower traces) in closed loop mode (B) Control Calibration signals = 100 grams tension 0.5 sec duration

prevent the vocal cords from becoming slack during their abduction at inspiration The expiratory contractions, on the other hand, varied in magnitude, becoming more pronounced with decreases in the level of anesthesia (compare upper traces in Fig 3 C and 3 D) and reached maximal tension levels in some studies Although the level of anesthesia was controlled in these studies so that vocalization would not occur, expiratory contractions of the cricothyroid muscles prepared the animal for vocalization by adducting and lengthening the vocal folds Both muscles contracted similarly whether exhibiting biphasic activity (Fig 3 E) or only inspiratory activity (Fig 3 A)

Following these observations of normal muscle contractions one of the cricothyroid muscles was paralysed by transecting its nerve (Fig 3 F, lower trace arrow) With the nerve severed changes in tension during respiration were, of course, no longer exhibited by this muscle (Fig 3 G, lower trace) This paralysed muscle was designated the slave muscle and electrodes introduced into the muscle leading to the muscle stimulation device which was set in closed loop configuration with adequate gain so that the slave muscle could attain th maximal tension developed by the control normal muscle In Fig 3 G the device wa turned on midway through the trace (arrow and the resulting contractions of the slav muscle again appeared similar to those of it partner in both peak tension and waveform That is, contractions of the electrically stimulated slave muscle closely paralleled contrac tions of the homologous normal muscle durin the respiratory cycle Similar results were ob tained in all dogs studied

II *Tracking of Complex Contractions of Control Muscle by the Slave Muscle*

In this phase of the study, the ability of th slave muscle to track contractions of the con trol muscle was evaluated by inducing a wid variety of complex responses in the control muscle by direct electrical stimulation

A *Dynamic ranges of responses of control and slave muscles, fused tetanus and twitch*

1 *Fused tetanus.* Since most muscles con tract in a fused but graded manner, the ability of the slave muscle to follow the control muscle when undergoing fused tetani of vary ing amplitudes was determined Since the ac curacy of the slave muscle response could be expected to be better with feedback, the de vice was set in closed loop configuration with as much feedback as possible without creatin uncontrolled oscillations in slave muscle re sponses This was accomplished by settin both exciter amplifier gains (G_c, G_s) high bu equal (see Fig 1) The device gain was ini tially set (by adjusting the stimulator powe amplifier gain G_p) so that the slave muscl matched the control muscle's plateau tensio during a maximum fused tetanus G_s was the reduced until no oscillations were apparent i the slave muscle response, G_p was reduce concomitantly so that the slave muscle coul still match the maximum control muscle ter sion plateau Once suitable gains for G_c, G_s

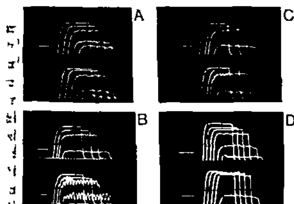


Fig 5 Effects of varying the magnitude of feedback on slave muscle tracking accuracy and stability (A) Closed loop mode $G_s<G_c$ (B) Closed loop mode $G_s>G_c$ (C) Closed loop mode, G_s slightly less than G_c (D) Open-loop mode $G_s=0$ *In all frames upper traces are control muscle tensions and lower traces are slave muscle tensions* Calibration signals = 100 grams tension 0 5 sec duration

and G_p were found, they were held constant and the ability of the slave muscle to track the control muscle throughout its dynamic range determined

The control muscle exhibited successively diminishing responses as the stimulus to it was reduced in intensity (Fig 4 A, upper traces, from left to right) The slave muscle (lower traces) closely tracked the control muscle in every case (and in all studied dogs) with only small aberrations in plateau tension near threshold As a control, the device was turned off in several instances during maximal stimulation of the control muscle As shown by example in Fig 4 B, in such instances the slave transducer recorded no change in tension, demonstrating that there was no mechanical linkage between the control muscle and the slave transducer

The effects of varying the magnitude of feedback on slave muscle response are shown in Fig 5 The amount of feedback in Fig 5 A was similar to that employed in the study illustrated in Fig 4 A, note again there were no uncontrolled oscillations in the slave muscle responses Any irregularity in waveform at a slave muscle tension plateau was a reflection of similar irregularity in waveform at the corresponding control muscle plateau In Fig 5 B the slave exciter amplifier gain (G_s) was increased so that it was larger than the control exciter amplifier gain (G_c) and uncontrolled oscillations occurred in the slave muscle responses When G_s was reduced so that it was slightly less than G_c, resonance still occurred in most studies, particularly at intermediate plateau tensions (Fig 5 C)

In Fig 5 D the feedback was reduced to zero, $G_s=0$, and the open loop mode obtained As expected, no oscillations occurred in the slave muscle responses, but the ability of the slave muscle to achieve plateau tensions equal to the control muscle throughout its dynamic range was compromised Responses of the slave muscle tended to be maximal or minimal, with overshooting or undershooting of intermediate plateau levels of the control muscle A further limitation of the device in open-loop mode was that responses of the slave muscle often varied from one trial

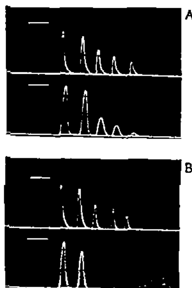


Fig 6 (A) Tracking of control muscle twitch tensions (upper traces) by the slave muscle (lower traces) in closed-loop mode (B) Tracking of control muscle twitch tensions by the slave muscle in open-loop mode The device gains used here for best matching of twitch tensions (in each mode) were higher than those used for best matching of plateau tensions of the two m

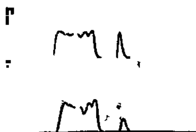


Fig 7 Demonstration of the dependency of the slave muscle response upon the duration of the control muscle contraction When the device gain was adjusted so that the slave muscle (left lower trace) could match a particular plateau tension of the control muscle (left upper trace) in open loop mode the slave muscle (right lower trace) could not match a control muscle twitch (right upper trace) equal in magnitude to the plateaus Calibration signals (far left) = 100 grams 0 5 sec duration

to the next without a change in control muscle tension input to the device

2 *Twitch* When the control muscle was stimulated by a single square-wave pulse, it twitched, reaching a tension peak which depended upon the strength of stimulus (Fig 6A, B, upper traces, decreasing stimulus strength from left to right) The slave muscle, however, appeared less capable of matching tensions of control muscle twitches in either device mode than it was in matching tensions of control muscle plateaus This point was demonstrated by increasing the device gain slightly so that the slave muscle could perfectly match a control muscle plateau, under such a condition, the slave muscle did not also match a control muscle twitch equal in magnitude to the plateaus (Fig 7) The device did not generate as great a response in the slave muscle over the shorter time interval of a twitch Although the number of slave muscle fibers recruited by the device stimulus would be the same for a control muscle twitch and plateau of equal tension, these recruited fibers may not have had sufficient time to tetanize and realize their maximum possible tensions during a twitch [1]

When G_p was increased to establish a new reference level for the slave muscle equal to the maximum twitch tension of the control muscle, the slave muscle was accurate in matching control muscle peak tension throughout its dynamic range when the device was in closed-loop (Fig 6A, lower trace) On the other hand, the slave muscle was somewhat inaccurate when an open loop mode was used As was observed for plateau tensions slave muscle twitches tended to be maximal or minimal with the intermediate peak tensions of the control muscle absent (Fig 6B, lower trace) When G_p was increased further in an effort to boost up lower peak tensions of the slave muscle to match those of the control muscle, higher peak tensions of the slave muscle were also boosted up, above those of the control muscle, resulting in no overall improvement in the slave muscle tracking ability

The duration of the slave muscle twitch was consistently greater than that of the control muscle, the contraction time or time-to peak being longer for the slave muscle (see Fig 6) The difference in contraction times was obviously related to the different manner in which the control and slave muscles were stimulated fibers of the control muscle were stimulated by a single square-wave pulse of almost instantaneous rise time while the slave muscle fibers were stimulated by a train of 2 to 3 square-waves whose amplitude (envelope) rose more slowly, with the rise in control muscle tension

B *Relative frequency responses of control and slave muscles*

A greater appreciation of the capabilities and limitations of the muscle stimulation device would result from knowledge of the relative frequency responses of control and slave muscles A muscle, such as the control muscle stimulated by constant voltage square-waves is limited in its ability to exhibit constant peak

[1] If the contraction time (time to peak) of the control muscle was 50 msec and the recruited slave muscle fibers were stimulated at 50 pulses/second they would receive cathodal pulses every 20 msec or 2 to 3 pulses over the 50 msec rise time

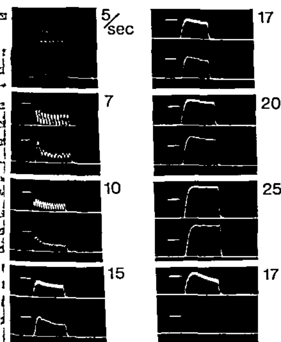


Fig 8 Relative frequency responses of the control muscle (upper traces) and the slave muscle (lower traces) in closed loop mode The frequency of stimulation of the control muscle is indicated to the right of each frame No significant differences were observed in the slave muscle frequency response when an open loop mode was used However frequent adjustment of the device gain was required in this mode to match the tension plateaus of the two muscles as the rate of stimulation of the control muscle was varied In one instance the muscle stimulation device was turned off as a control during stimulation of the control muscle (at 17 pulses/second) Calibration signals = 100 grams 0 5 sec duration

to peak tension excursions when the frequency of stimulation is increased In the study illustrated in Fig 8 the control muscle experienced a drop in peak to peak tension when the rate of stimulation was increased to 10 pulses/second (upper traces) At this frequency the period of the stimulus was less than the duration of the control muscle twitch so that partial fusion of twitches with formation of an unfused tetanus plateau occurred Further reduction in the magnitude of tension oscillation was apparent with increasing frequency of stimulation Whether the slave muscle would track the control muscle exhibiting the same reductions in peak to peak tension with increasing frequency of stimulation or whether further limitations in frequency response would arise was of interest

The slave muscle accurately tracked the control muscle when stimulated at a frequency of 5 pulses/second (Fig 8 lower traces) However, at a stimulation rate of 7 pulses/second premature fusion of slave muscle twitches was observed resulting in a reduction in the magnitude of tension oscillation below that observed for the control muscle The greater contraction time and twitch duration of the slave muscle was undoubtedly responsible for the fusion of slave muscle twitches at this lower rate of stimulation At higher rates of control muscle stimulation slave muscle tension oscillations were also smaller than those of the control muscle as a consequence of the premature fusion of slave muscle twitches

Another factor which may have been, in part, responsible for the smaller slave muscle tension oscillations about unfused tetanus plateaus was the relative inability of the slave muscle to match short duration tension changes of the control muscle when the device gain was set for matching their plateau tensions In studies such as the one illustrated in Fig 8 G_p was adjusted freely so that the average tension or plateau tension of the slave muscle equalled that of the control muscle the device would then recruit fully tetanized slave muscle fibers, primarily to form a tension plateau equal to that of the control muscle and incompletely tetanized fibers secondarily to produce tension oscillation about the plateau which was smaller in magnitude than control muscle oscillation

DISCUSSION

These studies indicate that it is feasible to consider chronic stimulation of a paralysed axial muscle such as the cricothyroid by a device as described to restore its natural function In all studies the paralysed slave cricothyroid muscles could be made to track natural nerve induced contractions of their normal contralateral homologous muscl



Fig 7 Demonstration of the dependency of the slave muscle response upon the duration of the control muscle contraction When the device gain was adjusted so that the slave muscle (left lower trace) could match a part ... on of the control muscle (left upper ...) in open loop mode the slave muscle (right lower trace) could not match a control muscle twitch (right upper trace) equal in magnitude to the plateaus Calibration signals (far left) = 100 grams 0 5 sec duration

to the next without a change in control muscle tension input to the device

2 *Twitch* When the control muscle was stimulated by a single square wave pulse it twitched reaching a tension peak which depended upon the strength of stimulus (Fig 6A B upper traces decreasing stimulus strength from left to right) The slave muscle however appeared less capable of matching tensions of control muscle twitches in either device mode than it was in matching tensions of control muscle plateaus This *point* was demonstrated by increasing the device *gain* slightly so that the slave muscle *could* perfectly match a control muscle plateau *under* such a condition the slave muscle *did not* also match a control muscle twitch equal in magnitude to the plateaus (Fig 7) The device did not generate as great a response in the slave muscle over the shorter time interval of a twitch Although the number of slave muscle fibers recruited by the device stimulus would be the same for a control muscle twitch and plateau of equal tension, these recruited fibers may not have had sufficient time to tetanize and realize their maximum possible tensions during a twitch [3]

When G_p was increased to establish a new reference level for the slave muscle equal to the maximum twitch tension of the control muscle the slave muscle was accurate in matching control muscle peak tensions throughout its dynamic range when the device was in closed loop (Fig 6A lower trace) On the other hand the slave muscle was somewhat inaccurate when an open loop mode was used As was observed for plateau tension slave muscle twitches tended to be maximal or minimal with the intermediate peak tensions of the control muscle absent (Fig 6B lower trace) When G_p was increased further in an effort to boost up lower peak tensions of the slave muscle to match those of the control muscle higher peak tensions of the slave muscle were also boosted up above those of the control muscle resulting in no overall improvement in the slave muscle trackability

The duration of the slave muscle twitch was consistently greater than that of the control muscle the contraction time or time to-peak being longer for the slave muscle (see Fig 6) The difference in contraction times was obviously related to the different manner in which the control and slave muscles were stimulated fibers of the control muscle were stimulated by a single square wave pulse of almost instantaneous rise time while the slave muscle fibers were stimulated by a train of 2 to 3 square waves whose amplitude (envelope) rose more slowly with the rise in control muscle tension

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[3] If the contraction time (time to peak) of the control muscle was 50 msec and the recruited slave muscle fibers were stimulated at 50 pulses/second they would receive cathodal pulses every 20 msec or 2 to 3 pulses over the 50 msec rise time

ies the open loop device might be suitable for implantation in some axial muscles, such as those controlling facial movements (e g eye-blink, smile) where accuracy is not of great importance

These studies demonstrate also the superiority of a closed loop system over an open-loop system When the device was in closed-loop mode, slave muscles accurately tracked their control muscles in various states of contraction (see Figs 4A, 5A, 6A) Furthermore, slave muscles were more consistent in their responses to a given control muscle tension input to the device in closed loop mode The explanation for a slave muscle's greater accuracy and consistency in tracking its control muscle rests in the closed loop system's ability to compensate for any slight changes in loop gain and, in particular, changes in slave "muscle gain" (see Appendix) through its error signal It was noted in these studies that slave muscle responses were most accurate when the feedback was high However, if the feedback (and feedback loop gain) was sufficiently large, unstable oscillations appeared at slave muscle tension plateaus These observations are to be expected in that feedback control theory predicts that sluggish systems with high feedback loop gains will be unstable (Di Stefano et al, 1967, Jewett & Rayner, 1976) A rigorous analysis of the conditions under which stability would be expected in this feedback system will be deferred to a later paper, it need only be stated that feedback conditions could be empirically found in all studies which bestowed accurate and stable responses to the slave muscles

In light of the present sophistication of electronic devices used in humans (pacemakers (Greatbatch et al 1965) prosthetic devices (Allen & Karchak, 1970 Dorcas et al, 1970)), functional neuromuscular stimulation (FNS) devices for stimulation of not only limb muscles (Mortimer & Peckham 1972, Van Der Meulen et al 1974, Vodovnik et al, 1972 Waters, 1972) but also axial muscles may eventually have clinical significance in human medicine Implantation of a muscle stimulation device as described could potentially result in complete recovery of all involuntary and voluntary functions of a paralysed axial muscle, since the control signal for the device is derived from an analogous muscle, the paralysed muscle's homologue These studies suggest that the recovered functions would also have the necessary precision However, there are many other factors which must be considered in implanting such a device for chronic stimulation The transducers used must reflect the contractile states of the implanted muscles whether contracting isometrically or isotonically They must not in any manner disrupt the normal actions of the implanted muscles, and must continue to function indefinitely once implanted At present there are no transducers available which satisfy all these requirements Electrodes which have been successfully used for chronic stimulation of human muscles are helically-coiled Caldwell (Caldwell & Reswick, 1967) type electrodes which flex with a muscle during contraction (Van Der Meulen et al, 1974) Although noble metals are preferred as electrode materials, stainless steel electrodes have been found to better tolerate repetitive flexion without breaking Unfortunately, rectification occurs with stainless steel electrodes (higher electrode impedance being observed during the anodal phase of the stimulus cycle Weinman 1965), so that the type of stimulus used will largely determine the extent of tissue damage and electrode corrosion In general it is believed that a biphasic voltage waveform (Lilly 1961) which is made charge symmetric (to minimize electrochemical damage of tissue) by adjusting the duration and shape of each phase of the cycle (to minimize electrode corrosion) should be used with stainless steel electrodes (Brummer & Turner 1972, Greatbatch et al 1965, Weinman 1965) Finally the frequency of stimulation is an important factor to be considered in chronic stimulation of an axial muscle Low stimulus frequencies (5–10 pulses/second) are desirable, because high f

quency stimulation can lead to failure of neuromuscular transmission (Krnjevic & Miledi 1958) and occlusion of the blood flow to a muscle (Folkow & Halicka 1968) both of which increase neuromuscular fatigue Furthermore chronic stimulation of a muscle with low frequencies can increase the fatigue resistance of a muscle by preventing or reversing the (disuse) atrophy of slow twitch intermediate muscle fibers and by converting fast twitch white muscle fibers to fast twitch red muscle fibers (Barnard et al 1970 1971 Buller et al 1960 Mortimer 1974 Van Der Meulen 1972 Van Der Meulen et al 1974) If the frequencies found suitable for stimulation are subfusion frequencies smooth contractions of a muscle can be obtained by a program of sequential stimulation (asynchronous stimulation of different regions of the muscle Peckham et al 1970) $T_s$max (the maximum slave muscle tension which can be produced) may also be reduced when using subfusion frequencies in which case consideration should be given to modulating not only the amplitude but the frequency of stimulation of the paralysed slave muscle Factors such as these are important in the development of a system for implantation and chronic stimulation of paralysed axial muscles in experimental animals If such a system is developed and found successful consideration could then be made for implantation in human patients

APPENDIX

The slave muscle was limited in its accuracy in open loop mode in tracking control muscle contractions of various magnitudes This tracking inaccuracy can be explained simply by the changes in the slave muscle voltage-tension relationship which occurred with changes in control muscle tension For example a plot of control muscle plateau tensions (T_c) and slave muscle plateau tensions (T_s) against the slave muscle stimulus voltage (V_s) from the study illustrated in Fig 5 D indicates that the slave muscle tracking inaccuracy stemmed from the nonlinear or changing relationship between T_s and V_s (Fig 9 A) Control muscle tension was linearly related to stimulus voltage with a slope (T_c/V_s) determined by the device gain (V_s/T_c) At points of intersection of the two curves the slave muscle equalled the control muscle tension because the ratio T_s/V_s (the slave muscle gain) equalled the control muscle curve slope or was reciprocally related to the device gain (i e $T_s/V_s \times V_s/T_c = 1$) Indeed the muscle gain times the device gain is the total open loop gain (T_s/T_c) and must equal unity in order for the tensions to be equal At other points on the slave muscle curve the muscl gain (T_s/V_s) changed Since the device gai was held constant it no longer bore a rec procal relationship to the muscle gain th open loop gain deviated from unity and th slave muscle did not accurately track the cor trol muscle Obviously the tracking accurac of the slave muscle could be improved if th two curves were made to coincide Since th slave muscle voltage-tension relationship car not be manipulated the accuracy could be im proved if the control muscle curve were mad to coincide with the slave muscle curve Thi could be accomplished by using a nonlinea device whose device gain varies inversely t the muscle gain Alternatively a linear devic as described could be used if stimulation of th slave muscle were restricted to voltages withi the linear region of its voltage tension curve Arguments similar to those presented in th paragraph can explain the observed inaccu racy of a slave muscle in tracking twitches o its control muscle in open loop mode (Fig 9 C)

The slave muscle was further limited in it accuracy in open loop mode in attaining ten sions of the control muscle contractions wher they varied in duration because changes in the slave muscle voltage tension relationship oc curred with changes in the duration as well as the magnitude of control muscle contractions If the control muscle of Fig 9 A underwent



Fig 10 Time dependency of slave muscle responses A stimulus of 1 36 V was delivered to the slave muscle by introducing a 200 gram tension step to the muscle stimulation device As the duration of the tension step (and stimulus) decreased in duration (1 000 500 200 100 20 5 msec from right to left upper traces) the slave muscle response became successively smaller (from right to left lower traces) Maximum slave muscle response=200 grams

shorter duration contractions such as twitches, the slave muscle stimulus also decreased in duration and the slave muscle voltage–tension curve shifted towards the abscissa so that the tension produced by the slave muscle (and the muscle gain) at any given stimulus voltage was proportionally smaller (compare the slave muscle curves in Fig 9A, B) Furthermore, Fig 10 demonstrates that successive reductions in the slave muscle response would occur at any given stimulus voltage following decreases of the duration in steps, so that actually a family of such nonlinear slave muscle voltage–tension curves exists in which those curves representing the shortest durations are closest to the abscissa The possible explanation for reduced slave muscle responses with decreases in duration was mentioned previously As the control muscle contraction and slave muscle stimulus decreased in duration the number of slave muscle fibers recruited by the stimulus would remain the same (if the stimulus amplitude were unchanged) but the recruited fibers may not have had time to fully tetanize and realize their maximum possible tensions Because of the shift of the slave muscle voltage–tension curve in Fig 9, the slave muscle was less capable of matching twitch tensions of the control muscle (Fig 9B), than it was in matching plateau tensions of the control muscle (Fig 9A) with the same device gain (the control muscle curve slopes are equal in Fig 9A, B) However, the difference in tracking accuracy was only significant at higher stimulus voltage where deviation between the two slave muscle curves increases Little difference in tracking accuracy was noted in studies such as the one illustrated in Fig 9B, because only small stimulus voltages and threshold slave muscle twitches could be evoked by the control muscle The control muscle twitch maximum $T_c$max, was only 90 grams [1] On the other hand a significant reduction in the tracking accuracy of a slave muscle might be encountered if its control muscle underwent natural twitches, since natural nerve induced twitches would probably have a larger $T_c$max (broken line, Fig 9B) than the electrically induced control muscle twitches of Fig 9B (i e during natural contractions including twitches, both the number of active muscle fibers and their rate of discharge increase so that recruited fibers are tetanized (Ganong, 1975), unlike the recruited muscle fibers of the control muscle of Fig 9B which were untetanized, stimulated by a single square wave pulse) However axial muscles do not ordinarily exhibit twitches Thus a shift in the slave muscle s voltage–tension curve with a decrease in duration as observed in these studies and its affect in limiting the tracking accuracy of the slave muscle has little practical significance A single voltage–tension curve similar to the one shown in Fig 9A would sufficiently describe the stimulus–response characteristics of a slave muscle for any natural contraction of its control muscle

[1] A significant difference in the slave muscle s abil ty to track plateaus and twitches was noticed in these studies by increas ng the device gain (see Fig 7)

SUMMARY

1 Paralysed slave cricothyroid muscles co be made to track natural , nerve ind

contractions and more complex, electrically induced contractions of their controlling homologues when stimulated by a muscle stimulation device as described However, the accuracy of the slave muscles in matching tensions of their control muscles depended upon the device mode used

2 In open loop mode slave muscle responses were inaccurate because the slave muscle voltage–tension relationships were nonlinear, time dependent, and somewhat inconsistent in a given preparation The time dependency is probably of little practical importance, since natural contractions of axial muscles are usually of longer duration than twitches Inaccuracy due to the nonlinearity could be reduced by restricting stimulation of the slave muscle to the linear region of its stimulus–response curve or by using a nonlinear device which complements the curve

3 In closed loop mode with the appropriate feedback loop gain, accurate, stable, and consistent responses could be obtained by slave muscles in tracking their control muscles

4 Many factors, such as the types of transducers electrodes, and stimuli which would be used, must be considered in developing a system for implantation and chronic stimulation of paralysed axial muscles

ACKNOWLEDGEMENTS

Special thanks go to Mike Merzenich and Lindsay Aitkin for their help in editing this manuscript to Gus Winston Mark White and Ed Mentzer for help in the design and construction of the muscle stimulation device and to Leona Wayrynen for typing this manuscript This paper is dedicated to the memory of Sam Zealear

ZUSAMMENFASSUNG

Man kann sich vorstellen daß die Funktion eines gelähmten axialen Muskels (auch Kehlkopf Gesichtsmuskeln oder extraokulare Muskeln) wiederhergestellt werden kann wenn man denselben wieder zum Kontrahieren bringen könnte genau wie seine Gegenseite Ein Gerät zur Muskelanregung wurde entworfen und konstruiert so daß die Anregung die man auf einen bestimmten gelähmten Muskel anwandte durch ein Signal reguliert wurde das den kontrahierenden Zustand der Gegenseite reflektierte Studien in Kehlkopfmuskeln von Hunden weisen darauf hin daß gelähmte Muskeln die von einem solchen offenen Schleifengerät (*open-loop device*) angeregt wurden ihre Gegenseite imitieren konnten Doch die nachweisbare Genauigkeit der Experimente war durch die Wechselbeziehung der Anregungs Reaktion eingeschränkt Auf der anderen Seite wurde weit größere Genauigkeit nachgewiesen wenn ein geschlossenes Schleifengerät (*closed-loop device*) angewendet wurde *das heißt wenn auch Rückwirkungs Information (feed* back information) von den angeregten Muskeln gebraucht wurde um den Grad der Anregung zu kontrollieren Es wird in Erwägung gezogen solche Apparate (offene Schleifen oder geschlossene Schleifengeräte) bei gelähmten Muskeln zur dauernden Anregung einzusetzen

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EXPERIMENTAL HEMIGLOSSECTOMY WITH AN ARGON LASER ON TONGUES FROM DEAD HUMANS AND OF LIVE RABBITS

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Abstract On 8 tongues of deceased humans and 2 live rabbit tongues drilling and incision experiments were performed using an argon laser With a power of 50 W a hem glossectomy was performed in 3–4 min The average thickness of the tongue was 2 cm and the incision length 13 cm It was shown on the live rabb t tongue that no bleed ng occurs during a partial hemiglossectomy except at the arteria lingualis The macroscopical and histolog ical changes of the tissue are described viz the carboni zation zones the irreparable cell damage and the coagula tion and the hyperaemic edge The cutting speeds and the incision and drill ng data for tongues of varying th ckness provide informat on on the techn cal conditions for clin ical applicat on In addition temperatures in the tissue during incis on were recorded The advantages of laser surgery of the tongue are compared with those achieved with the electric kn fe and scalpel

The purpose of this work was to carry out experimental surgery on the tongue in the form of hemiglossectomy using a high power argon laser It has been shown by many authors (Fox 1974 Goodale et al 1970 Kaplan et al 1973, Mussiggang & Katsaros 1971 Mussig gang & Rother 1974 Stellar et al 1970) that cutting the tissue with a focused laser beam is an almost bloodless operation Bleeding oc curs only when large vessels are severed Surgical experiments on the tongue of live rabbits were carried out to show to what ex tent bloodless laser surgery is possible even in the case of hemiglossectomy Bloodless sur gery is important, especially on the tongue which is supplied very intensively with blood

It is possible that laser surgery in the form of hemiglossectomy offers surgical advantages compared with conventional methods using scalpel or electric knife

The first step towards laser surgery is to determine the laser power necessary for cutting the tissue In addition the cutting velocity has to be established It will be shown that the present level of technology in this field provides lasers of sufficient power for carrying out a hemiglossectomy on the human tongue

MATERIALS AND METHODS

Eight tongues of recently deceased humans of both sexes (1–6 days post mortem 61–81 years of age preserved at 1°C) were used Further experiments were performed on the tongues of two live rabbits under anesthesia An argon laser constructed by the Technical University of Berlin (Schafer & Seelig 1970) was available with a power of 1–80 W in multimode operation The wavelengths were 0 488 μm and 0 514 μm (blue green) of almost equal power The beam diameter was 12 mm with a divergence angle of 10^{-3} rad The beam was focused through a quartz lens with a focal length of 20 cm Three thermocouples (chromium–nickel wires 0 1 mm in diameter) served for temperature measurements and a one and two channel recorder were used

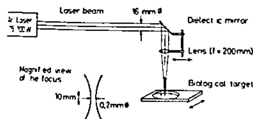


Fig 1 Experimental arrangement

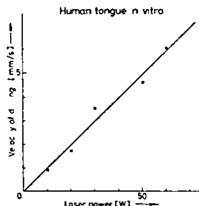


Fig 2 Drilling speed in relation to laser power in respect of the human tongue

Procedure

A human tongue larynx preparation was obtained by dissection It was put on a plate, which could be moved uniformly in a horizontal plane (Fig 1) Traction sutures were applied on both sides of the tongue to hold the tongue in the correct position The laser beam was focused on the tongue by means of a mirror and a lens (Fig 1) The focal length chosen was $f = 20$ cm, giving a focus of 0 2 mm diameter The beam was about 5% greater in diameter at about ±5 mm out of focus The focus was adjusted at a lower laser power (50 W) to about 1 cm under the surface of the tongue Then the beam was brought up to the edge of the tip of the tongue and the laser power of 50 W was switched on by deblocking the laser resonator with a magnetic flap The plate was moved horizontally at the velocity necessary to completely cut the tongue down the centre axis Both halves of the tongue were held to the side by traction sutures When the base of the tongue was reached it was pushed laterally to the laser beam to complete the hemiglossectomy The time taken to cut through the tongue, the thickness of the severed tongue section and the length of the incision were measured Six hemiglossectomies were performed in this way Three thermocouples were pushed into the tissue about 8 mm under the surface to measure the temperatures occurring within the region of the tongue when operating with the laser beam The distances from the edge were 0 8 2 0 and 2 5 mm The thermocouples were connected up to recorders and the temperature curves registered during the laser surgery

For the drilling experiments, two tongues were incised down the centre axis with a scalpel and the laser beam focused about 2 mm away from the edge of the incision The tongues were perforated at laser powers of 10–60 W The thickness of the tongues in the region of the incision was determined About 40 perforations were made and measured

Experiments were carried out to determine the occurrence of bleeding Two rabbits were anesthetized intravenously with pentobarbital–natrium at a dosage rate of 1 ml per kg body weight to try out bloodless operation in the case of hemiglossectomy The animals were placed in supine position with head sharply reclining on the movable plate The mouth was opened and the jaws were fixed with a mouth gag The tongue was drawn forward and two traction sutures were applied to hold it in the required position The palate was protected from the laser radiation by a piece of asbestos which was introduced into the oral cavity The laser beam was focused on the back of the tongue Two hemiglossectomies were performed with a power of 50 W The length of the incision was limited by the length the tongue could be drawn out of the mouth

In all experiments specimens of the tissue surrounding the laser incision were taken and fixed in formaldehyde (5%) These specimens



Fig 3 Drilling experiments on the lengthwise severed dead human tongue (↗)=Drilling canals at a distance of 2 mm from the cut surface of the tongue (A+B) (A) Laser beam entering the tongue (↓) (B) The laser beam has completely penetrated the tongue (↓)=beam entering the surface of the tongue (↙)=emerging beam (←) beam shining through the tissue (C) Longitudinal section through a drilled canal (see A and B) showing complete carbonization (↗) (D) Light microscopical cross section of the drilled canals (see A and B) with the carbonization and coagulation zone (↑) (E) Cross section D at a higher magnification

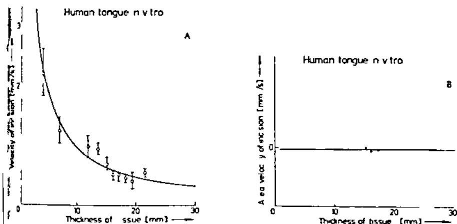


Fig 4 (A) Cutting speed of the tongue related to the thickness (laser power 50 W) (B) Area velocity calculated from (A) related to the thickness of the tissue The result is a constant value

in Paraplast Slices were obtained which were stained with haematoxylin and eosin and examined under a microscope

RESULTS

1 *Laser drilling of the human tongue in vitro*

Immediately after switching on the laser beam at a power above 10 W the tissue in focus begins to vaporize and burn In all experiments the tongue was perforated completely The drilling velocity is shown in Fig 2 in relation to the laser power The holes were produced about 2 mm from the incised centre axis of the tongue (Fig 3 A) While the laser beam travelled through the tongue the incised surface of the tissue was bright red and nothing unusual could be detected macroscopically afterwards (Fig 3 A–C) The macroscopic study of a longitudinal section through the drilled hole revealed a circular zone of carbonization (Fig 3 D) A histological picture shows an additional coagulation zone (Fig 3 E)

2 *Laser incision of the human tongue in vitro*

The laser beam is able to incise the thin tip of the tongue more rapidly than the thick centre portion The various incision velocities related to the thickness of the tissue are shown in Fig 4 in respect of a laser power of 50 W The cutting velocity which is calculated from Fig 4 A is shown in Fig 4 B This gives constant value for the incision surface per unit of time of about 10 mm²/sec

The time required for a total hemiglossectomy was 3–4 minutes the length of the incision being 12–13 cm and the average thickness 1 5–2 cm (Fig 5 A)

During the incision operation a small tissue stria with a thickness corresponding to the diameter of the drilled holes is vaporized and burned Macroscopically the incised surface shows a carbonisation zone (Fig 5 A B) The histological study also reveals an area with coagulated cells (Fig 5 C, D)

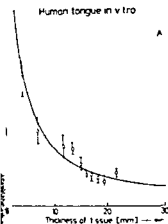
Temperatures of some 2 000°C occur during the transection (one thermocouple even melted) The temperature curve shows a rapid drop in temperature from the incised edge into the tissue (Fig 6 A) At about 2 5 mm from the edge the temperature rise was only about 40°C above normal body temperature The temperature in relation to time is shown in Fig 6 B

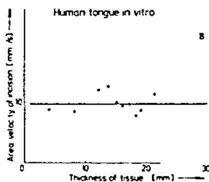
3 *Laser incision of the rabbit tongue in vivo*

Immediately the laser beam is focused on the tongue the tissue vaporizes and the incision is made very rapidly The incision surfaces are blackened and free of blood (Fig 5 B) Onl



Fig 3 Drilling experiments on the lengthwise severed dead human tongue (↗) Drilling canals at a distance of 2 mm from the cut surface of the tongue (A+B) (A) Laser beam entering the tongue (↓) (B) The laser beam has completely penetrated the tongue (↓) beam entering the surface of the tongue (↙)=emerging beam (←) beam shining through the tissue (C) Longitudinal sectio through a drilled canal (see A and B) showing complet carbonization (↗) (D) Light microscopical cross sectic of the drilled canals (see A and B) with the carbonizatio and coagulation zone (↑) (E) Cross section D at a high magnification





4 (A) Cutting speed of the tongue related to the (laser power 50 W) (B) Area velocity calculated from (A) related to the thickness of the tissue The result is a constant value

last Slices were obtained which were with haematoxylin and eosin and ex- a microscope

RESULTS

Laser drilling of the human tongue in vitro

mmediately after switching on the laser beam a power above 10 W the tissue in focus to vaporize and burn In all experi- the tongue was perforated completely he drilling velocity is shown in Fig 2 in re ation to the laser power The holes were about 2 mm from the incised centre is of the tongue (Fig 3 A) While the laser velled through the tongue the incised of the tissue was bright red and noth- unusual could be detected macroscopi- afterwards (Fig 3 A–C) The macro- scopic study of a longitudinal section through drilled hole revealed a circular zone of zation (Fig 3 D) A histological picture an additional coagulation zone (Fig E)

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Temperatures of some 2 000°C occur during the transection (one thermocouple even melted) The temperature curve shows a rapid drop in temperature from the incised edge into the tissue (Fig 6 A) At about 2 5 mm from the edge the temperature rise was only about 40°C above normal body temperature The temperature in relation to time is shown in Fig 6 B

3 *Laser incision of the rabbit tongue in vivo*

Immediately the laser beam is focused on the tongue the tissue vaporizes and the incision is made very rapidly The incision surfaces are blackened and free of blood (Fig 5 B) Only



Fig 5 State post laser hemiglossectomy of the dead human tongue (A+B) and the living rabbit tongue (C+D) (A+C) Macroscopical study (B+D)=The corresponding light microscopical sections of (A+C) The sectional planes are carbonized and sharply defined (A+C) and especially bloodless in (C) showing a small white coagulation zone (↓) on the surface of the tongue (B+D) A small sharply defined carbonization zone and coagulation can be seen (↑)

spurt bleeding occurred at the base of the tongue in both experiments due to the severing arteria lingualis vessels The hemorrhage was stanched easily by suturing after the part hemiglossectomy On studying the upper and lower side of the tongue macroscopically 1 mm thick whitish stria parallel with the carbonized edge of incision could be observed (Fig 5B) Five minutes later a reddish m zone was seen About 5–10 min after hemiglossectomy the half tongue and the tongue of the rabbit were slightly swollen and showed a distinct reddening The incised surfaces remained free of blood The length of the incision was 3–4 cm which was made in 30–40 sec The tongue was about 0 5 cm thick (Fig 5B)

The following zones would be observed histologically (Fig 5D) The carbonization area is bordered by a small zone of irreparable cell damage This is followed by a 2 mm broad stria with coagulation of the tubular epithelium which is adjoined by a small hyperaemic zone The demarcation from the healthy tissue is quite clear

DISCUSSION

The laser beam required to cut the human tongue must be as narrow as possible this being achieved by correct focusing The beam must not be allowed to spread to any great extent i e the focus had a large field of depth

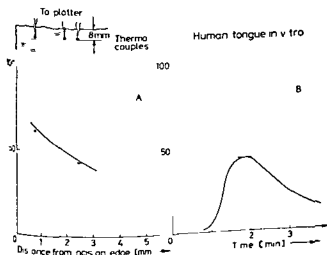


Fig 6 (*a*) Temperature distribution in the incised tongue according to the distance from the edge of the incision (laser power 50 W) (*b*) Temperature in relation to time at a distance of 2 5 mm from the cutting surface

Thus a focal length of 20 cm was chosen, giving an average power density of 2×10^5 W/cm^2 at a focal diameter of 0 2 mm and with a power of 50 W The power density is constant to within 10% at a field of depth of 1 cm

The drilling experiments provided information on the laser power necessary for incising the tongue Under the described focusing conditions a laser power of about 50 W provides cutting velocities which are reasonable for possible clinical application At this power rating a 2 cm thick tongue is perforated in 7 sec *After this period the beam has to be* moved, otherwise the tissue under the tongue will be damaged The time required for hemiglossectomy of the human tongue is about 3–4 min at 50 W This means that the technical conditions for clinical application can be fulfilled

In our experiments a rigid laser beam was used and the tissue was moved under the focal point For clinical application a practical beam manipulator which can be handled easily like a scalpel will be necessary (Kaplan et al , 1973, Mussiggang & Rother, 1974, Stellar et al , 1970) In this case sharper focusing with a smaller field of depth may be more expedient Consequently, it may be that laser ratings 50 W will be adequate for laser surgery the human tongue, especially if monomade , are used

The most interesting phenomenon of laser surgery is the lack of blood This phenomenon shows itself clearly on the vocal cords of dogs and in the treatment of various lesions of the human vocal cords (Hobeika & Rockwell 1972, 1973, Jako, 1967 Jako & Strong 1973, Strong & Jako 1972) Previous experiments have shown that small blood vessels become welded before the tissue is incised (Lenz & Eichler, 1975, Lenz et al , 1976) It may be that the argon laser is most suitable for bloodless surgery because the radiation is absorbed mainly by the blood However no detailed comparison of the hemostatic action of various types of laser has yet been published

Histology shows that the tissue near the edge of the incision is damaged at a dept about 2–3 mm This statement is supporte the temperature measurements At abo mm depth the maximum temperature of living tissue is about 70°C, which is slig higher than the critical temperature for denaturation of egg albumen (Chia Lu Barnes, 1970) Using a practical laser b and sharper focusing within the region o tissue, damage may be considerably less

Laser surgery of the tongue has the ad tage of being a bloodless and rapid proce *reduces the hazard of infection due to the* tactless procedure and the good closure o wound The contactless action ensures the tissue is not mechanically damaged does happen in electrosurgery when the tric knife is lifted clear of the wound thermore, there is a sharper demarcation tween healthy and damaged tissue when c pared with electrosurgery

ZUSAMMENFASSUNG

An 8 menschlichen Zungen von frisch Verstorbener an 2 lebenden Kaninchenzungen werden Durchbohr Schnittversuche mit einem Argon Laser in Form Hemiglossektomie durchgefuhrt Mit einer Leistun 50 Watt wird eine Hemiglossektomie bei einer mit Zungendicke von 2 cm und einer Schnittflachenlang 13 cm nach 3–4 min erzielt Die an lebenden K chenzungen durchgefuhrten Hemiglossektomien z die Blutlosigkeit des Vorgehens bis auf Blutungen a

ein hyperaemischer Randsaum Die für versch Zungendicken gemessenen Schnitt und Durch geschwindigkeiten geben *Anhaltspunkte für eine mö* klinische Anwendung des Argon Lasers bei der I glossektomie Ferner werden die wahrend der In auftretenden Temperaturen im Gewebe gemessen Vorte le der Laserchirurgie an der Zunge werde dem Vorgehen durch Elektrochirurgie und Skalpel glichen

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OSTIAL RESISTANCE

Its Variations and Correlations to the Patency Test Results

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(Received August 15 1976)

Abstract Resistance measurement is a simple and practical means of testing ostial function The present study showed that the results of measuring the ostial resistance varied according to the amount of antral secretion For that reason the postirrigational mean resistance is about 1/5 of the highest mean resistance in acute maxillary sinusitis The average ostial resistance in healthy persons is 2 5 cm of water and in acute maxillary sinusitis at *diagnostic puncture 13 0 cm of water and at the end of* treatment 3 3 cm of water There are discrepancies between the results of resistance measuring and patency tests in 10–30% of cases It is difficult to say whether these are due more to faults in the measuring techniques of the resistance or the patency As an ostial function test resistance measuring is quantitative and the testing of patency qualitative

g of ostial resistance has not gained *prominence in clinical practice which it* serves as a simple test of ostial function One reason for this may be the fact that results gained by this method have seldom been reported in the literature Another reason is that the results are not absolute but relative (Zippel & Meier, 1968)

Flottes et al (1960) used air in their resistance measurements Drettner (1965) and Zippel & Meier (1968) used saline In both techniques the pressure was produced by elevating at a slow, constant rate a bottle with irrigating fluid The bottle was connected to a puncture needle introduced into the maxillary sinus by tubing The ostial resistance i e the distance between the fluid level of the bottle and the ostium was read at the moment the fluid began to flow out of the nostril Results of both afore mentioned investigations were nearly identical The pressure increase in the antrum has also been produced by using a hand pump (Rantanen & Kortekangas, 197 *In this technique the overpressure in the bot* was read from a mercury manometer at the moment the saline began to flow in a dri chamber

Drettner (1965) observed that in acute maxillary sinusitis ostial resistance corresponds 10–25 cm of water in every second case but more elevated in the rest The results we similar when using the hand pump techniqu *in which the united resistance of the ostiu* and secretion before irrigation was measure but the resistance corresponded to normal 0 mmHg in about 70%, when only the postirrigational resistance probably produced by swelling of the mucosa was taken into accou (Rantanen 1974) On recovery from sinusiti the return of the resistance to normal has bee demonstrated by all the above mentioned investigators

The purpose of this study was firstly to corroborate these wide variations which occur the results of resistance measuring second to determine the average ostial resistance healthy persons and in acute maxillar sinusitis and thirdly to study discrepancies b

Table I *Ostial resistance*

	Resistance (mmHg)								Mean resist ance	No of sinuses
	0	1–5	6–15	16–30	31–50	51–100	101–300	>300		
Sinusitis series										
At diagnostic puncture										
Highest resistance	3	30	29	12	11	16	12	5	45 3	
Resistance after irrig	10	48	37	16	5	1	1		9 7	/118
During treatment										
Highest resistance	5	38	13	7	9	4	14		36 4	
Resistance after irrig	8	54	15	5	8				6 6	/90
At end of treatment										
Highest resistance	24	73	7	5	1	3	5		11 7	
Resistance after irrig	27	85	3	2	1				2 4	/118
Healthy persons										
At diagnostic puncture										
Highest resistance	7	44							1 8	
Resistance after irrig	7	44							1 8	/51

ween the results of resistance measuring and atency testing

MATERIAL AND METHODS

e material was the same as that in my earlier tudy (1974) The sinusitis series consisted of 18 acute, untreated maxillary sinusitis cases vith retention of secretion, and a control eries of 51 sinuses in healthy persons The echniques of resistance measuring and pa ency testing have both been described in letail in the above mentioned study In re- istance measuring the hand pump and drip hamber technique was used The patency ests were carried out by using the consecutive measuring technique

The resistance was measured in four phases at each examination immediately after punc ture of the maxillary sinus—the so called primary resistance and immediately before as well as after irrigating the sinus with 100 ml 0 9% saline Because a non homogeneous sinus secretion will cause variations in measur ing results, the greatest ostial resistance during he measuring was also determined called the highest ostial resistance in this study The re sistance measurements were repeated weekly until recovery, in all cases

The ostial respiratory patency was determined after irrigating the sinus According to the patency the ostia were categorized as open, partially open, or obstructed The ostial sniff penetrance was also tested after irriga tion

RESULTS

The highest ostial resistances and the re- sistances after irrigation are presented in Table I From this table it can be seen that the post irrigational mean resistance was about 1/5 of the highest mean resistance at all stages of sinusitis at diagnostic puncture, dur- ing treatment, and at the end of treatment

The primary resistance was identical with the highest resistance in 40 8% (133/326) but less in all the other cases The highest re sistance appeared primarily only in 15 0% (20/133) at diagnostic puncture but in 56 4% (75/133) at the end of treatment

The highest ostial resistance was identical with the resistance before irrigation in 59 2% (193/326) out of which 28 9% (56/194) oc- curred at diagnostic puncture and 47 4% (92/ 194) at the end of treatment

The resistance was higher before irrigation than after it in 38 0% (124/326) and less i

Table II *Ostial resistance and patency at post irrigational examination*

Ostium	Resistance (mmHg) 0	1	2	3	4	5	>5	Mean resistance	No of sinus
Open	18	30	25	13	5	2	2	1 7	95
Partially open	16	19	17	16	1	3	15	3 2	87
Obstructed	10	20	12	10	6	3	83	14 2	144
Sinuses	44	69	54	39	12	8	100		326

6 8% (22/326) In all the other cases these resistances appeared identical in 32 2% (58/180) at diagnostic puncture and in 43 9% (85/180) at the end of treatment

In healthy persons the highest ostial resistance was identical with the resistance after irrigation in all 51 cases the mean resistance being 1 8 mmHg

When comparing the resistance with the respiratory patency the resistance was more than 1 mmHg, i e more than 13 6 mm H_2O in 47 cases with an open ostium, although the average inspiratory pressure decrease during quiet nasal respiration inside the sinus was 14 8 mm H_2O (Table II) In an obstructed ostium the resistance was ≤1 mmHg, i e less than the mean inspiratory pressure decrease in 30 cases In all these above mentioned 77 cases (32 2%) there occurred a discrepancy -tween the results of the resistance meas- and patency test In sinuses with an ostium the resistance should have been ≤1 mmHg in all cases and in an obstructed ostium >1 mmHg in all cases

A corresponding discrepancy occurred in 9 3% in 22 cases when comparing the results of the post irrigational resistance and sniff penetrance (Table III) in ostia with normal penetrance the resistance was more thar mmHg in 13 cases and in 9 cases less tha mmHg, although the sniffing produced pressure change inside the sinus

DISCUSSION

There are in clinical practice two differ techniques in which the ostial resistance measured by using fluid to elevate the ant pressure Both methods have their vantages It is preferable to raise a bot with irrigation fluid at a slow, constant rate means of an elevator, as did Drettner (196 and Zippel & Meier (1968) The antral pre sure increases evenly, whereas the increase uneven when a hand pump is used The pre sion of the results is also better, when resu of the resistance are read from the distan between the fluid level of the bottle and t infra orbital margin, than by reading the ant overpressure values from a mercury manom ter

In the present study the highest ostial sistance was, in about 11% of the cases, mo than 100 mmHg, corresponding to a distar of over 136 cm between the fluid level a

Table III *Ostial resistance and sniff penetrance at post irrigational examination*

Penetrance (mm H_2O)	Resistance (mmHg) 0	1	2	3	4	5	6–15	>15	Mean resistance	No of sinus
>60	40	53	40	28	7	5	13		2 0	186
60–1	2	11	11	9	5	2	29	21	15 1	90
0	1	5	2			1	19	22	17 8	50
Sinuses	43	69	53	37	12	8	61	43		326

-orbital margin It is not practical to raise bottle so far above the margin

n the present study too, a drip-chamber d as an indicator to indicate the moment at h the fluid began to flow into the sinus and ostium opened The moment at which the d or the sinus secretion begins to issue from nasal canal depends on the antral volume, ntity of the antral secretion and the degree ostial obstruction When the antrum is e, the relative quantity of the sinus secre- small, or the ostium obstructed, the re- ance values will be inaccurate, when they read at the moment the fluid begins to flow of the nostril, as it always takes a little time re the secretion or irrigation fluid is in the tril This is a disadvantage which can be inated by using a drip-chamber as an indi r in ostial resistance measuring

he normal restistance varies in healthy ons from 10 to 25 cm of water (Zippel & er, 1968) When a drip chamber was used n indicator in measuring corresponding es were 0–5 mmHg, i e 0–7 cm of water present study also showed that in healthy ons both the highest mean ostial re- ance and the resistance after irrigation are mmHg, corresponding to 2 5 cm of water the other hand in all phases of acute sitis, the combined resistance of the um and secretion is five times as high as ostial mean resistance after irrigation, n the sinus is free of secretion This wide ation in the measuring results is due to etion, because of which the highest ostial stance is found as early as at the beginning he resistance measuring in 40% of cases

his study also showed that the primary stance is simultaneously the highest ostial ce more frequently at the end of treat- at diagnostic puncture and also that resistances before and after irrigation are often identical at the end of treatment at diagnostic puncture The writer's opin is that these differences are attributable to swelling in the ostial canal and to the fact the secretion at the end of treatment is more mucous and homogeneous than at diagnostic puncture, on which occasion the highest ostial resistance can appear at any time during resistance measurement The above-mentioned differences can also be a sign of deterioration in the ciliar function during the early stage of an infection, because of which in such a case the secretion lies at the bottom of the sinus and only moves when the antral pressure increases during the resistance measuring

There were discrepancies between the results in 32% when comparing the ostial resistance and the ostial respiratory patency and in 9% when the resistance was compared with the ostial sniff penetrance These discrepancies, as for instance ostial resistance >40 cm of water in an open or partially open ostium, or ostial resistance 0–27 cm of water in an obstructed ostium Corresponding observations about the results of resistance measurement and penetrance test may be due to either of the ostial function tests There are many conceivable reasons for these discrepancies in the patency tests the peak pressure values fluctuate continuously during respiration and sniffing, even in the same person leading to incorrect evaluation results concerning the ostial respiratory patency On the other hand it must be remembered that the patency tests are qualitative, whereas the measuring of the resistance is a quantitative ostial function test This means in practice that resistance measuring is more suitable than patency testing in cases when ostia are obstructed In addition to these sources of error it is possible in both the ostial function tests to obtain incorrect results owing to technical matters It is therefore very difficult to decide, whether these errors are more common in the patency tests or in the resistance measurements

ZUSAMMENFASSUNG

Die Messung der Resistenz ist eine einfache und praktische Methode die Funktion des Ostiums der Kieferhöhle zu untersuchen Diese Untersuchung ergab Resultat der Resistenzmessung viel variiert und d

nation am meisten vom Sekret resultiert Aus diesem Grunde ist die Resistenz des Ostiums nach der Irrigation der Kieferhöhle nur 1/5 von der höchsten Resistenz desselben Ostiums in akuten Kieferhöhlenentzündungen Die durchschnittliche Resistenz des Ostiums ist 2 5 cm Wasser in gesunden Personen und 13 0 cm Wasser am Anfang und 3 3 cm Wasser zum Ende der Kieferhöhlenentzündung Die Streitigkeiten zwischen den Resultaten der Resistenzmessung und der Durchgängigkeitstesten, in 10–30 Prozent, resultieren von den teknischen Fehlern in beiden Untersuchungsmethoden Die Messung der Resistenz ist als eine quantitative die Teste der Durchgängigkeit qualitative Methode zu betrachten die Funktion des Kieferhöhlenostiums zu untersuchen

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